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ABSTRACTS

Volume 8, Part 2

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CONTENTS

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CHRONOLOGICAL LIST OF SESSIONS

(See page vi for Thematic List of Sessions.)

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Session Number and Title **Page**

8:30 AM Friday

Symposia

Slide Sessions

Poster Sessions

Sessions end at 12:45 PM, Friday, November 5.

THEMATIC LIST OF SESSIONS*

Theme A: Development and Plasticity

Session Number	Title	Type	Day	Time
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70.	Cell Lineage and Differentiation I	Slide	Tue	8:30 AM
179.	Cell Lineage and Differentiation II	Poster	Wed	1:00 PM
9.	Development of Invertebrates I	Slide	Mon	8:30 AM
178.	Development of Invertebrates II	Poster	Wed	1:00 PM
122.	Development and Plasticity: Aging	Poster	Tue	1:00 PM
6.	Development and Plasticity: Autonomic Nervous System I	Slide	Mon	8:30 AM
52.	Development and Plasticity: Autonomic Nervous System II	Poster	Mon	1:00 PM
49.	Development and Plasticity: Biochemical and Pharmacological Correlates of Development	Poster	Mon	1:00 PM
168.	Development and Plasticity: Developmental Disorders	Poster	Wed	8:30 AM
89.	Development and Plasticity: Limbic System	Poster	Tue	8:30 AM
23.	Development and Plasticity: Neurotoxicology	Poster	Mon	8:30 AM
50.	Development and Plasticity: Nutritional and Prenatal Factors	Poster	Mon	1:00 PM
142.	Development and Plasticity: Retinotectal Connections	Slide	Wed	8:30 AM
216.	Development and Plasticity: Sensory Systems	Poster	Thu	8:30 AM
53.	Development and Plasticity: Trophic Agents I	Poster	Mon	1:00 PM
110.	Development and Plasticity: Trophic Agents II	Slide	Tue	1:00 PM
51.	Development and Plasticity: Trophic Interactions	Poster	Mon	1:00 PM
190.	Developmental Effects of Activity	Poster	Wed	1:00 PM
217.	Effects of Denervation	Poster	Thu	8:30 AM
54.	Endocrine Control of Development I	Poster	Mon	1:00 PM
249.	Endocrine Control of Development II	Poster	Thu	1:00 PM
180.	Morphogenesis and Pattern Formation I	Poster	Wed	1:00 PM
235.	Morphogenesis and Pattern Formation II	Slide	Thu	1:00 PM
277.	Motor System Development	Poster	Fri	8:30 AM
40.	Neural Plasticity in Adult Animals I	Slide	Mon	1:00 PM
215.	Neural Plasticity in Adult Animals II	Poster	Thu	8:30 AM
181.	Neuronal Death and Synapse Elimination	Poster	Wed	1:00 PM
262.	Regeneration in the Central Nervous System	Slide	Fri	8:30 AM
218.	Regeneration in the Central Nervous System: Forebrain Pathways	Poster	Thu	8:30 AM
248.	Regeneration in the Central Nervous System: Ventral Nerve Cord and Spinal Pathways	Poster	Thu	1:00 PM
12.	Regeneration in the Peripheral Nervous System I	Slide	Mon	8:30 AM
246.	Regeneration in the Peripheral Nervous System II	Poster	Thu	1:00 PM
233.	Retinogeniculate Development	Slide	Thu	1:00 PM
125.	Retinotectal Development and Organization	Poster	Tue	1:00 PM
205.	Role of Activity in Synaptic Sorting and Elimination	Slide	Thu	8:30 AM
121.	Specificity of Synaptic Connections	Poster	Tue	1:00 PM
84.	Sprouting and Sprouting Mechanisms	Poster	Tue	8:30 AM
247.	Synaptogenesis	Poster	Thu	1:00 PM
36.	Synaptogenesis: Molecular Approaches	Slide	Mon	1:00 PM
5.	Visual System: Geniculocortical Development I	Slide	Mon	8:30 AM
82.	Visual System: Geniculocortical Development II	Poster	Tue	8:30 AM
189.	Visual System: Geniculocortical Development III	Poster	Wed	1:00 PM

*Includes contributed paper sessions, symposia, and workshops only.

Theme B: Cell Biology

Session Number	Title	Type	Day	Time
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283.	Cell Biology: Metabolic Studies	Poster	Fri	8:30 AM
197.	Cell Surface Macromolecules, Receptors, and Membranes	Poster	Wed	1:00 PM
62.	Cell and Tissue Culture	Poster	Mon	1:00 PM
286.	Cellular Aspects of Disease	Poster	Fri	8:30 AM
63.	Glia: Morphology and Function	Poster	Mon	1:00 PM
196.	Identified Neurons	Poster	Wed	1:00 PM
207.	Identifying Neurons and Glial Cells	Slide	Thu	8:30 AM
116.	Membranes and Cell Surface Molecules	Slide	Tue	1:00 PM
203.	Neurogenetics and Gene Expression	Slide	Thu	8:30 AM
64.	Neuronal and Glial Macromolecules	Poster	Mon	1:00 PM
148.	Structure and Function of Neuroendocrine Cells	Slide	Wed	8:30 AM

Theme C: Excitable Membranes and Synaptic Transmission

Session Number	Title	Type	Day	Time
169.	Diseases of Synapses and Axons	Poster	Wed	8:30 AM
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115.	Electrophysiological Behavior of Vertebrate Central Nervous System Neurons	Slide	Tue	1:00 PM
287.	Epilepsy	Poster	Fri	8:30 AM
194.	Invertebrate Neurobiology	Poster	Wed	1:00 PM
66.	Ionic Channel Mechanisms	Poster	Mon	1:00 PM
211.	Ionic Channels: Structure and Function	Slide	Thu	8:30 AM
33.	Ionic Mechanisms of Excitable Cells	Poster	Mon	8:30 AM
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271.	Membrane Biophysics II	Slide	Fri	8:30 AM
227.	Neurobiological Studies of Cells in Culture	Poster	Thu	8:30 AM
259.	Neurobiological Studies of Vertebrate Central Nervous System Neurons	Poster	Thu	1:00 PM
101.	Pharmacology of Synaptic Transmission: Central Nervous System	Poster	Tue	8:30 AM
226.	Postsynaptic Mechanisms: Central Nervous System	Poster	Thu	8:30 AM
135.	Postsynaptic Mechanisms: Peripheral Nervous System	Poster	Tue	1:00 PM
145.	Pre- and Postsynaptic Mechanisms I	Slide	Wed	8:30 AM
239.	Pre- and Postsynaptic Mechanisms II	Slide	Thu	1:00 PM
225.	Presynaptic Mechanisms: Central Nervous System	Poster	Thu	8:30 AM
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258.	Sensory Transduction	Poster	Thu	1:00 PM
77.	Synaptic Structure and Function I	Slide	Tue	8:30 AM
133.	Synaptic Structure and Function II	Poster	Tue	1:00 PM
104.	Molecular and Genetic Studies of the Voltage-Dependent Sodium Channel	Symp.	Tue	1:00 PM
171.	Single Channel Recording	Symp.	Wed	1:00 PM

Theme D: Neurotransmitters, Modulators, and Receptors

Session Number	Title	Type	Day	Time
143.	Acetylcholine I	Slide	Wed	8:30 AM
221.	Acetylcholine II	Poster	Thu	8:30 AM
157.	Adenosine: Modulators	Poster	Wed	8:30 AM
146.	Adrenergic Receptors: Biochemistry I	Slide	Wed	8:30 AM
187.	Adrenergic Receptors: Biochemistry II	Poster	Wed	1:00 PM
185.	Alcohol II	Poster	Wed	1:00 PM
111.	Amino Acids	Slide	Tue	1:00 PM
27.	Behavioral Pharmacology I	Poster	Mon	8:30 AM
109.	Behavioral Pharmacology II	Slide	Tue	1:00 PM
28.	Behavioral Pharmacology: Sedatives and Anxiolytics	Poster	Mon	8:30 AM
158.	Benzodiazepines	Poster	Wed	8:30 AM
223.	Biogenic Amines: Phenethylamine, Tryptamine, Serotonin, and Histamine	Poster	Thu	8:30 AM
30.	Catecholamines: Anatomical Localization	Poster	Mon	8:30 AM
252.	Catecholamines: Biochemistry	Poster	Thu	1:00 PM
131.	Catecholamines: Physiological Effects I	Poster	Tue	1:00 PM
264.	Catecholamines: Physiological Effects II	Slide	Fri	8:30 AM
31.	Coexistence of Transmitters	Poster	Mon	8:30 AM
93.	Cyclic Nucleotides	Poster	Tue	8:30 AM
186.	Dopamine Receptors: Biochemistry I	Poster	Wed	1:00 PM
208.	Dopamine Receptors: Biochemistry II	Slide	Thu	8:30 AM
251.	Excitatory Amino Acids	Poster	Thu	1:00 PM
159.	GABA and Benzodiazepines	Poster	Wed	8:30 AM
29.	Hypothalamic Hormones: Anatomical Localization	Poster	Mon	8:30 AM
32.	Interaction Between Neurotransmitters	Poster	Mon	8:30 AM
92.	Muscarinic Receptors	Poster	Tue	8:30 AM
91.	Nicotinic Receptors	Poster	Tue	8:30 AM
26.	Opiates, Endorphins, and Enkephalins: Anatomical Localization	Poster	Mon	8:30 AM
130.	Opiates, Endorphins, and Enkephalins: Characterization, Biosynthesis, and Degradation	Poster	Tue	1:00 PM
61.	Opiates, Endorphins, and Enkephalins: Electrophysiological Effects	Poster	Mon	1:00 PM
60.	Opiates, Endorphins, and Enkephalins: Physiological Effects I	Poster	Mon	1:00 PM
222.	Opiates, Endorphins, and Enkephalins: Physiological Effects II	Poster	Thu	8:30 AM
59.	Opiates, Endorphins, and Enkephalins: Receptors I	Poster	Mon	1:00 PM
107.	Opiates, Endorphins, and Enkephalins: Receptors II	Slide	Tue	1:00 PM
160.	Peptide-Amine Interactions: Modulators	Poster	Wed	8:30 AM
161.	Peptides: Anatomical Localization I	Poster	Wed	8:30 AM
231.	Peptides: Anatomical Localization II	Slide	Thu	1:00 PM
8.	Peptides: Biochemical Characterization	Slide	Mon	8:30 AM
79.	Peptides: Physiological Effects I	Slide	Tue	8:30 AM
281.	Peptides: Physiological Effects II	Poster	Fri	8:30 AM
280.	Peptides: Receptors	Poster	Fri	8:30 AM
156.	Phosphorylation: Calmodulin and Calcium Channels	Poster	Wed	8:30 AM
10.	Receptor Localization and Characterization I	Slide	Mon	8:30 AM
183.	Receptor Localization and Characterization II	Poster	Wed	1:00 PM
234.	Regulation of Transmitter Metabolic Enzymes	Slide	Thu	1:00 PM
76.	Serotonin: Biochemistry and Physiology	Slide	Tue	8:30 AM
38.	Transmitter Cytochemistry and Immunohistochemistry I	Slide	Mon	1:00 PM
188.	Transmitter Cytochemistry and Immunohistochemistry II	Poster	Wed	1:00 PM
282.	Transmitters in Invertebrates	Poster	Fri	8:30 AM
44.	Transmitters and Receptors in Disease I	Slide	Mon	1:00 PM
155.	Transmitters and Receptors in Disease II	Poster	Wed	8:30 AM
132.	Uptake, Storage, and Secretion I	Poster	Tue	1:00 PM
144.	Uptake, Storage, and Secretion II	Slide	Wed	8:30 AM
184.	Uptake, Storage, and Secretion: Cholinergic Systems	Poster	Wed	1:00 PM
260.	Functions of Multiple Transmitters in Neurons	Symp.	Fri	8:30 AM
201.	Substance P as a Neurotransmitter	Symp.	Thu	8:30 AM
35.	Sympathetic Ganglia as Models for Synaptic Transmitter Action	Symp.	Mon	1:00 PM

Theme E: Endocrine and Autonomic Regulation

Session Number	Title	Type	Day	Time
88.	Brain Ventricular Systems	Poster	Tue	8:30 AM
73.	Cardiovascular Regulation: Central Transmitters I	Slide	Tue	8:30 AM
119.	Cardiovascular Regulation: Central Transmitters II	Poster	Tue	1:00 PM
21.	Cardiovascular Regulation: Functional Aspects I	Poster	Mon	8:30 AM
209.	Cardiovascular Regulation: Functional Aspects II	Slide	Thu	8:30 AM
120.	Cardiovascular Regulation: Hypertension and Stress	Poster	Tue	1:00 PM
39.	Endocrine and Autonomic Regulation: Central Pathways I	Slide	Mon	1:00 PM
118.	Endocrine and Autonomic Regulation: Central Pathways II	Poster	Tue	1:00 PM
19.	Hormonal Control of Behavior I	Poster	Mon	8:30 AM
267.	Hormonal Control of Behavior II	Slide	Fri	8:30 AM
20.	Neural Control of Immune System	Poster	Mon	8:30 AM
113.	Peripheral Autonomic Nervous System I	Slide	Tue	1:00 PM
153.	Peripheral Autonomic Nervous System II	Poster	Wed	8:30 AM
152.	Pineal Gland	Poster	Wed	8:30 AM
22.	Regulation of Autonomic Functions I	Poster	Mon	8:30 AM
74.	Regulation of Autonomic Functions II	Slide	Tue	8:30 AM
18.	Regulation of Pituitary Function	Poster	Mon	8:30 AM
154.	Respiratory Regulation	Poster	Wed	8:30 AM

Theme F: Sensory Systems

Session Number	Title	Type	Day	Time
288.	Auditory Cortex	Poster	Fri	8:30 AM
16.	Auditory and Vestibular Sensory Organs	Poster	Mon	8:30 AM
7.	Chemical Senses: Olfaction and Taste I	Slide	Mon	8:30 AM
55.	Chemical Senses: Olfaction and Taste II	Poster	Mon	1:00 PM
95.	Evoked Potentials I	Poster	Tue	8:30 AM
265.	Evoked Potentials II	Slide	Fri	8:30 AM
72.	Pain I	Slide	Tue	8:30 AM
220.	Pain: Modulation I	Poster	Thu	8:30 AM
230.	Pain: Modulation II	Slide	Thu	1:00 PM
17.	Retina: Intrinsic Organization I	Poster	Mon	8:30 AM
37.	Retina: Intrinsic Organization II	Slide	Mon	1:00 PM
244.	Sensory System: Somatosensory Cortex	Poster	Thu	1:00 PM
147.	Sensory Systems in Invertebrates I	Slide	Wed	8:30 AM
195.	Sensory Systems in Invertebrates II	Poster	Wed	1:00 PM
245.	Skin, Muscle, and Visceral Receptors	Poster	Thu	1:00 PM
15.	Somatosensory Cortex and Thalamus	Slide	Mon	8:30 AM
25.	Spinal Cord I	Poster	Mon	8:30 AM
43.	Subcortical Auditory Pathways I	Slide	Mon	1:00 PM
94.	Subcortical Auditory Pathways II	Poster	Tue	8:30 AM
289.	Subcortical Somatosensory Pathways	Poster	Fri	8:30 AM
56.	Subcortical Visual Pathways I	Poster	Mon	1:00 PM
71.	Subcortical Visual Pathways II	Slide	Tue	8:30 AM
112.	Subcortical Visual Pathways III	Slide	Tue	1:00 PM
129.	Transmitters in Sensory Systems	Poster	Tue	1:00 PM
191.	Visual Cortex: Cortical and Subcortical Relationships	Poster	Wed	1:00 PM
193.	Visual Cortex: Extrastriate Visual Areas I	Poster	Wed	1:00 PM
232.	Visual Cortex: Extrastriate Visual Areas II	Slide	Thu	1:00 PM
192.	Visual Cortex: Intrinsic Organization of Striate Cortex I	Poster	Wed	1:00 PM
204.	Visual Cortex: Intrinsic Organization of Striate Cortex II	Slide	Thu	8:30 AM
138.	Functions of Extrastriate Visual Cortex in Primates	Symp.	Wed	8:30 AM
69.	Physiological Insights Derived from Nerve Traffic Analysis in Conscious Man	Symp.	Tue	8:30 AM

Theme G: Motor Systems and Sensorimotor Integration

Session Number	Title	Type	Day	Time
140.	Basal Ganglia	Slide	Wed	8:30 AM
276.	Basal Ganglia: Functional Relationships	Poster	Fri	8:30 AM
48.	Basal Ganglia: Structural Relationships	Poster	Mon	1:00 PM
123.	Cerebellum I	Poster	Tue	1:00 PM
238.	Cerebellum II	Slide	Thu	1:00 PM
47.	Control of Posture and Movement I	Poster	Mon	1:00 PM
78.	Control of Posture and Movement II	Slide	Tue	8:30 AM
212.	Control of Posture and Movement III	Poster	Thu	8:30 AM
272.	Control of Posture and Movement IV	Poster	Fri	8:30 AM
274.	Disorders of the Motor System and Corrective Methods	Poster	Fri	8:30 AM
213.	Invertebrate Motor Function	Poster	Thu	8:30 AM
114.	Motor Systems: Cortex I	Slide	Tue	1:00 PM
150.	Motor Systems: Cortex II	Poster	Wed	8:30 AM
210.	Motor Systems: Spinal Cord and Brainstem I	Slide	Thu	8:30 AM
250.	Motor Systems: Spinal Cord and Brainstem II	Poster	Thu	1:00 PM
275.	Motor Systems: Spinal Cord and Brainstem III	Poster	Fri	8:30 AM
90.	Muscle	Poster	Tue	8:30 AM
124.	Muscle Afferents	Poster	Tue	1:00 PM
45.	Oculomotor I	Slide	Mon	1:00 PM
80.	Oculomotor II	Poster	Tue	8:30 AM
117.	Oculomotor III	Poster	Tue	1:00 PM
149.	Reflex Function	Poster	Wed	8:30 AM
273.	Sensorimotor Integration	Poster	Fri	8:30 AM
198.	Vestibular System	Poster	Wed	1:00 PM
269.	Vestibular System and Vestibulo-Ocular Reflex	Slide	Fri	8:30 AM
81.	Visuomotor Integration	Poster	Tue	8:30 AM
173.	Springing into Action: Mechanism and Function of Spring-Like Properties of Neuromuscular Systems	Wkshp.	Wed	1:00 PM
4.	Theoretical Approaches to Motor Control	Symp.	Mon	8:30 AM

Theme H: Structure and Function of the CNS

Session Number	Title	Type	Day	Time
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219.	Comparative Neuroanatomy	Poster	Thu	8:30 AM
57.	Cortex and Cortico-Subcortical Relationships I	Poster	Mon	1:00 PM
268.	Cortex and Cortico-Subcortical Relationships II	Slide	Fri	8:30 AM
13.	Diseases of the Central Nervous System I	Slide	Mon	8:30 AM
65.	Diseases of the Central Nervous System II	Poster	Mon	1:00 PM
24.	Epilepsy: Kindling	Poster	Mon	8:30 AM
126.	Epilepsy: Kindling and Pharmacology	Poster	Tue	1:00 PM
139.	Epilepsy: Pharmacology	Slide	Wed	8:30 AM
279.	Evoked Potentials and EEG	Poster	Fri	8:30 AM
240.	Limbic System and Hypothalamus I	Slide	Thu	1:00 PM
278.	Limbic System and Hypothalamus II	Poster	Fri	8:30 AM
58.	Limbic System and Hypothalamus: Structure	Poster	Mon	1:00 PM
284.	Spinal Cord II	Poster	Fri	8:30 AM
182.	Subcortical Organization	Poster	Wed	1:00 PM
202.	Application of Video Enhancement and Intensification Techniques to Neurobiology	Wkshp.	Thu	8:30 AM
229.	Functional Correlates of Brain Transplantation	Symp.	Thu	1:00 PM

Theme I: Neural Basis of Behavior

Session Number	Title	Type	Day	Time
241.	Aging	Poster	Thu	1:00 PM
163.	Alcohol I	Poster	Wed	8:30 AM
256.	Angiotensin and Drinking	Poster	Thu	1:00 PM
166.	Behavior and Learning in Invertebrate and Simple Vertebrate Preparations	Poster	Wed	8:30 AM
14.	Biological Rhythms I	Slide	Mon	8:30 AM
151.	Biological Rhythms II	Poster	Wed	8:30 AM
46.	Circuitry and Pattern Generation I	Slide	Mon	1:00 PM
214.	Circuitry and Pattern Generation II	Poster	Thu	8:30 AM
290.	Drugs of Abuse	Poster	Fri	8:30 AM
96.	Effects of Chronic Drug Administration and Neurotoxicology	Poster	Tue	8:30 AM
164.	Feeding and Drinking	Poster	Wed	8:30 AM
206.	Feeding and Drinking: Central Mechanisms and Neuropharmacology	Slide	Thu	8:30 AM
75.	Feeding and Drinking: Central and Peripheral Mechanisms	Slide	Tue	8:30 AM
255.	Feeding and Drinking: Cues for Need State	Poster	Thu	1:00 PM
257.	Feeding and Drinking: Metabolic Aspects	Poster	Thu	1:00 PM
165.	Feeding and Drinking: Neuropharmacology	Poster	Wed	8:30 AM
263.	Human Behavioral Neurobiology	Slide	Fri	8:30 AM
177.	Human Neuropsychology	Poster	Wed	1:00 PM
176.	Interhemispheric Relations	Poster	Wed	1:00 PM
106.	Invertebrate Learning and Behavior I	Slide	Tue	1:00 PM
236.	Invertebrate Learning and Behavior II	Slide	Thu	1:00 PM
42.	Learning and Memory: Electrophysiological and Pharmacological Studies	Slide	Mon	1:00 PM
86.	Learning and Memory: Electrophysiological Studies	Poster	Tue	8:30 AM
11.	Learning and Memory: Lesion Studies I	Slide	Mon	8:30 AM
85.	Learning and Memory: Lesion Studies II	Poster	Tue	8:30 AM
87.	Learning and Memory: Pharmacological Studies	Poster	Tue	8:30 AM
108.	Monoamines and Behavior	Slide	Tue	1:00 PM
97.	Monoamines and Behavior: Chronic Administration and Long-Lasting Effects	Poster	Tue	8:30 AM
98.	Monoamines and Behavior: Human Mental Disorders and Animal Models	Poster	Tue	8:30 AM
253.	Monoamines and Behavior: Movement and Reflexes	Poster	Thu	1:00 PM
254.	Monoamines and Behavior: Unit Recording Studies and Self-Stimulation Studies	Poster	Thu	1:00 PM
175.	Motivation and Emotion	Poster	Wed	1:00 PM
167.	Neuroethology I	Poster	Wed	8:30 AM
270.	Neuroethology II	Slide	Fri	8:30 AM
41.	Neuropeptides and Behavior I	Slide	Mon	1:00 PM
100.	Neuropeptides and Behavior II	Poster	Tue	8:30 AM
99.	Neuropeptides and Learned Behavior	Poster	Tue	8:30 AM
162.	Opiates, Endorphins, and Enkephalins	Poster	Wed	8:30 AM
141.	Pain II	Slide	Wed	8:30 AM
174.	Pain III	Poster	Wed	1:00 PM
128.	Psychotherapeutic Drugs	Poster	Tue	1:00 PM
242.	Sleep	Poster	Thu	1:00 PM
127.	Stress, Hormones, and the Autonomic Nervous System	Poster	Tue	1:00 PM
170.	Alzheimer's Disease and the Cholinergic Innervation of Neocortex by the Nucleus Basalis	Symp.	Wed	1:00 PM
228.	Neurobiology of Anxiety	Symp.	Thu	1:00 PM
261.	Neurobiology of Feeding Behavior	Symp.	Fri	8:30 AM
105.	Primate Frontal Lobes: Mechanisms for the Ordering of Complex Behavior	Symp.	Tue	1:00 PM

137 SYMPOSIUM. GENETIC CORRELATES OF MENTAL DISEASE. S. Matthysse*, McLean Hospital/Harvard Medical School (Chairman), L. Heston*, Univ. of Minnesota, S.S. Kety, McLean Hospital/Harvard Medical School, K.K. Kidd*, Yale Univ. Medical School.

This symposium will review the state of knowledge in the genetics of affective disorder, schizophrenia and Alzheimer's disease. New genetic methods, including restriction endonuclease polymorphisms, will be discussed. Current diagnostic practices will be examined in the light of genetic knowledge.

Brief summary of individual topics:

Dr. Heston - family studies of Alzheimer's disease, frequency of familial recurrence, comparison of familial and non-familial cases.

Dr. Kety - current status of the genetics of schizophrenia and schizophrenia spectrum illnesses.

Dr. Kidd - current status of the genetics of affective disorder, assessment of reports of genetic linkage, prospects for the use of restriction endonuclease polymorphisms for linkage analysis.

Dr. Matthysse - implications of genetic thinking for diagnostic classification.

Time will be reserved for open discussion.

138 SYMPOSIUM. FUNCTIONS OF EXTRA-STRIATE VISUAL CORTEX C.G. Gross, Princeton Univ. (Chairman); L.G. Ungerleider, Lab. of Neuropsychology, N.I.M.H.; C.G. Gross, Princeton Univ.; J.M. Allman, California Institute of Technology; R. Desimone*, Lab. of Neuropsychology, N.I.M.H.

The study of visual cortical mechanism currently faces an embarrassment of riches and a poverty of explanations. In the last decade, thanks initially to Allman and Kaas and to Zeki, there has been a population explosion of visual areas. Now there are at least a dozen visual areas in the cortex of cats and monkeys and more are coming. What are they all doing?

One type of visual cortex consists of the various prestriate areas, each of which contains a retinotopically organized representation of the contralateral visual field. Each of these areas seems to be specialized for analysis of different stimulus dimensions. Another type of visual cortex is inferior temporal (IT) cortex, which is not retinotopically organized. IT neurons usually have bilateral receptive fields and often show selectivity for complex stimuli.

Dr. Ungerleider will review the anatomical interconnections of various extra-striate areas and trace the flow of visual information through them. She will propose that there may be a dorsal occipito-parietal system crucial for the perception of spatial relations and a ventral occipito-temporal system crucial for object recognition and memory.

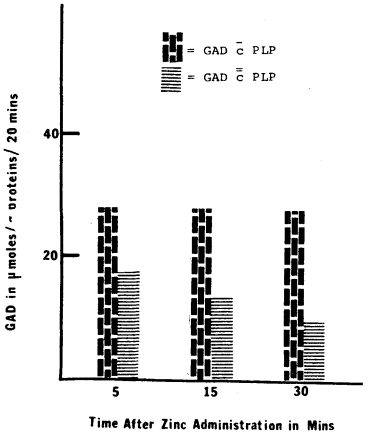
Dr. Gross will review the properties of IT, an area crucial for pattern recogniton. He will contrast IT with MT, an area involved in the processing of visual motion, and STP, a polysensory area in the temporal lobe.

Dr. Allman will discuss the analysis of motion and form by prestriate neurons, particularly in Areas MT and DL. He will suggest ways in which this analysis may underlie perceptual phenomena, such as discrimination of relative motion and of figure from ground.

Dr. Desimone will consider the filtering of chromatic and spatial information in Area V4 and will examine the possibility that inferior temporal neurons may act as shape filters.

139.1 THE NATURE AND MECHANISM OF ZINC-INDUCED EPILEPTIC SEIZURES. M. Ebadi, M. Itoh* and S. Swanson*. Dept. of Pharmacology, Univ. of Neb. Coll. of Med., Omaha, NE 68105.

The intracerebroventricular injection of $ZnCl_2$ in concentrations of 0.2 µmol/10 µl caused epileptic seizures rapidly characterized by rearing, running fits, jumping, retropulsion, vocalization, and convulsions. The convulsive episodes became manifested in fasiculation of facial muscles, jerking of head, myoclonic movements of the limbs, and sometimes, tonic-clonic convulsions. The latency periods between the administration of $ZnCl_2$ and the appearance of paroxysmal behaviors and movements were between 70 to 150 seconds. The ICV administration of GABA (0.4 µmol/10 µl) was able to block, prevent or rapidly reverse the Zn^{++}-induced epileptic seizures. When glutamic acid decarboxylase (GAD) was assayed in the absence of pyridoxal phosphate (PLP), the activities were significantly reduced in hippocampus with more pronounced effects seen after 30 mins incubation periods. The effect was specific for hippocampus but not other brain areas studied. The administration of $ZnSO_4$ did not alter the level of PLP in hippocampus, which has the highest concentration of Zn^{++} and the lowest level of PLP. The concentrations of PLP and Zn^{++} are negatively correlated (r = -0.53) in 14 regions of rat brain. Using Sephadex G-75 and Sephadex G-150 column chromatographies calibrated with proteins of known molecular weights, three separate zinc binding proteins with apparent estimated molecular weight of 210,000, 25,000 and 15,000 daltons respectively were detected in brain. The characteristics of these zinc binding proteins were not identical to hepatic or renal zinc-thioneins, since the administration of Zn^{++} did not increase their contents. Furthermore, comparative elution patterns showed that those peaks containing high activity of GAD possessed little or no zinc binding proteins. (Supported by a grant from Am. Epilepsy Fdn.)



139.2 PUSH-PULL PERFUSION OF PENTYLENETETRAZOL IN THE BRAIN STEM AND CEREBRAL CORTEX OF "ENCEPHALE ISOLE" CATS. F. Velasco, M. Velasco* and R. Romo* Sci. Res. Dept. Natl. Med. Ctr. IMSS - P.O. Box 73-032, México, D. F.

The role of the brain stem in the generation of pentylenetetrazol (PTZ) seizures was studied by perfusing the convulsant through a push-pull cannula in various places of the brain stem. Animals were immobilized in the stereotaxic frame by means of C2-C3 spinal cord transection and EEG from right and left motor cortices, EMG from neck muscles and ocular movements were recorded through implanted electrodes.

Perfusions from 0.2 to 1.0 ml of a solution containing 10 mg of PTZ/ml in the mesencephalic reticular formation (MRF), induced either myoclonic twitching of neck and head muscles and EEG interrupted discharges, or EEG sustained rhythmic discharges and muscular tonic clonic seizures, similar to those seen when PTZ is injected systemically. Perfusion of pontine reticular formation (PRF) induced EEG spindle bursts and muscular hypotonia. In other areas of the brain stem PTZ induced ocular movements in the form of tonic deviation or nystagmus, but no EEG or muscular seizures.

Similar perfusions of PTZ in the motor cortex induces focal EEG interrupted discharges with occasional muscular twitching, and in the suprasylvian cortex EEG interrupted discharges without clinical seizures.

Results suggest that PTZ has a differential effect in various structures of the CNS and that its convulsive properties result from its action on MRF.

139.3 THE EFFECT OF TAURINE ON ACUTE EPILEPTOGENIC FOCUS. D. Marcano de Cotte*, A. Alvarez*, J. R. Pérez*, M. A. Requena*, E. Vallecalle* and B. Drujan* (SPON: N. Wexler). Dept. of Physiology, J. M. Vargas Medical School, Universidad Central de Venezuela and Dept. of Neurochemistry, I.V.I.C., Apartado de Correos 47697, Caracas 1041, Venezuela.

Taurine is after glutamate the free amino acid with the great est concentration in the mature brain. It has been considered a putative inhibitory neurotransmitter in the central nervous system and retina. There is evidence that both human epilepsy and experimental epilepsy is accompanied by changes in the brain taurine concentrations. In animals with an experimental epileptic focus produced by application of cobalt to the brain surface, the site of the induced seizures activity shows lowered taurine levels. Repeated injections of taurine abolish seizure activity and reverse the abnormalities in brain amino acid levels which accompany severe epilepsy (Van Gelder, N. M., Brain Research, 47: 157, 1972).

The present study has been performed to investigate the effect of i.v. administration of taurine on the electrical activity of the epileptogenic focus induced by Penicillin applied on the right sensitive-motor cortex of adult Sprague Dawley rats. Taurine (100 mg/Kg body weight) was administered by the tail vein at 15, 30, 60 and 120 min. before the application of 1 mg of Sodium Penicillin G. The EEG was unipolarly recorded by means of silver ball electrodes applied to the pia and screw electrodes attached to the skull.

When taurine was administered 60 and 120 min before the application of penicillin, the delay of appearance of the focus was increased, the frecuency and spike amplitude decreased. The spread of epileptogenic focus to the opposite hemisphere was retarded when compared to control animals. Fifty per cent of the animals showed a reduced post-discharges frecuency. In 11 out 13 rats, taurine abolished the seizure activity completely.

In our experiments taurine is a powerful antiepileptic agent and the maximal antiepileptic effect was observed when the amino acid was administered 60-120 min. before the penicillin. This suggests that a better knowledge of the taurine metabolism is required to explain its antiepileptic activity.

This work was supported by a Grant from Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela.

139.4 TAURINE AND THE ONTOGENESIS OF AN EPILEPTOGENIC FOCUS IN THE RAT. A. Alvarez*, M. A. Requena*, E. Vallecalle*, D. Marcano de Cotte* and B. Drujan* (SPON: H. J. Finol). Dept. of Physiology, J. M. Vargas Medical School, Universidad Central de Venezuela and Dept. of Neurochemistry, I. V. I. C., Apartado de Correos 47697, Caracas 1041, Venezuela.

Previous reports have shown a consistent deficit of taurine in both epileptic human cerebral cortex and cobalt chronic cortical foci in cats and mice. Taurine is the free amino acid with the greatest concentration in the inmature brain. On the other hand, it has been shown that brain taurine in developing rats is derived from the mother, both in utero prior to birth and from the milk after birth.

In the present study we have investigated the effect of taurine administration to female Sprague Dawley rats before pregnancy, throughout gestation and throughout waning, on the development of EEG epileptic activity of the pups. Taurine (200 mg/Kg body weight) was administered intraperitoneally to the mothers and epilepsy was induced by application of 1 mg of Sodium Penicillin G on the right sensitive-motor cortex of pups at different post-natal ages. The EEG was unipolarly recorded by means of silver ball electrodes applied to the pia and screw electrodes attached to the skull.

Pups of 15, 18, 21 and 23 days of post-natal life did not show difference in the latency of appearance of the epileptic focus, spike amplitude and spike frecuency when compared to control animals. However, in the 50% of the animals which received taurine the epileptic activity remained localized to the application site and the other 50% showed a spread epileptic activity to the opposite sensitive-motor cortex. A spread to visual cortex was never observed. Furthermore, no post-discharges or generalized hypersynchronized activity was observed. These was significative different to control animals which always present post-discharges and, after the 21th day show seizure activity.

These results may suggest that the resistance of the most inmature brain (less of 15 days of post-natal life) to epileptic activity could be related to its high concentration of taurine.

Determination of brain taurine concentration in normal and epileptic animals from 1 to 30 days of age are being carried out.

This work was supported by a Grant from Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela.

139.5 BIOCHEMICAL CORRELATES OF THE ANTICONVULSANT ACTION OF TAURINE IN THE GENETICALLY EPILEPTIC RAT. Douglas Bonhaus*, Hugh Laird and Ryan J. Huxtable*. Department of Pharmacology, College of Medicine and Department of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, Tucson, Arizona 85721

Taurine (2-amino ethane sulfonic acid) is a potent anticonvulsant in a variety of experimental epilepsies. In the genetically epileptic rat, taurine has been shown to increase intracerebral electroshock thresholds and to decrease the intensity of sound induced seizures. The anticonvulsant action of taurine in this model is persistent. A single bilateral injection of 200 nMoles taurine into the inferior colliculi produced a statistically significant increase in intracerebral electroshock threshold for eighteen days. However, taurine did not alter intracerebral electroshock thresholds of control, genetically seizure resistant, rats (Huxtable, R. and Laird, H., Can. J. Neuro. Sci. 5: 215, 1978). The mechanism of the anticonvulsant action of taurine is not known. Few biochemical actions of taurine have been found that correlate with its anticonvulsant effect. Therefore, we find it interesting that taurine increases glutamic acid decarboxylase activity in tissue homogenates prepared from brains of epileptic rats, in as much as this enzyme produces gamma-aminobutyric acid, an inhibitory neurotransmitter. Taurine, in the presence of pyridoxine, increased glutamic acid decarboxylase activity in seizure susceptible rats to 126 ± 10 (mean ± S. E.) percent of control values ($p < 0.05$, paired Student's t test). Taurine is without effect on the glutamic acid decarboxylase activity of seizure resistant rats. These biochemical findings are intriguing, because they correlate with the differential effect of taurine on the electroshock threshold of the two strains. These findings may, therefore, offer some insight into the mechanism of the anticonvulsant action of taurine. (Supported by USPHS HL 19394).

139.6 PRENATAL EXPOSURE TO ELECTROCONVULSIVE SEIZURES, A BENZODIAZEPINE ANTAGONIST AND AN AGONIST: EFFECTS ON POSTNATAL DEVELOPMENT OF BENZODIAZEPINE BINDING AND SEIZURE THRESHOLDS. D.W. Gallager, Dept. Psychiat. and Sect. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06508.

Electroconvulsive seizures and the specific benzodiazepine antagonist, Ro15-1788, administered during the period of fetal benzodiazepine (BZ) binding site proliferation significantly decreased the density of cortical BZ sites in exposed offspring at various postnatal (PN) ages. Significant decreases in electroshock seizure threshold were observed in offspring following these prenatal treatments.

Total 3H-diazepam binding was decreased in cerebral cortical tissue from offspring following maximal electroshock seizures (MES) administered to pregnant rats on days 16, 18, and 20 in gestation. Scatchard analysis of membranes from MES-exposed and sham offspring showed that the decrease in specific 3H-diazepam binding was due to a decrease in the total number of binding sites without a significant change in affinity (Sham: KD = 4.2 nM, Bmax = 1419 fmol/mg protein; MES: KD = 4.4 nM, Bmax = 1292 fmol/mg protein). MES administered at other times in gestation did not result in significant BZ site alterations in exposed offspring. Decreases in electroshock seizure thresholds of MES-exposed offspring were also observed. Mean thresholds (measured at PN 14 through 17) were 9.5 ± 0.2 mA in control and 8.7 ± 0.1 mA in MES exposed offspring.

Subcutaneous administration of 20 mg/kg of the benzodiazepine antagonist Ro15-1788 to pregnant rats on days 14 through 17 in gestation also resulted in significant decreases in BZ site density as compared with offspring from control rats treated in parallel with vehicle (dilute Tween 80). Scatchard analysis of cortical membranes from offspring exposed prenatally to Ro15-1788 had a KD of 4.1 nM and a Bmax of 1155 fmol/mg protein as compared vehicle control offspring with a KD of 4.2 nM and a Bmax of 1345 fmol/mg protein. This prenatal treatment also resulted in significant decreases in seizure threshold. Mean electroshock seizure thresholds were 8.7 ± 0.2 mA for vehicle and 7.7 ± 0.2 mA for offspring exposed prenatally to Ro15-1788. In contrast, chronic prenatal treatment with the potent BZ agonist clonazepam (2 mg/kg s.c. daily to pregnant rats on days 14 through 20 in gestation) produced no significant alterations in BZ sites or seizure threshold in exposed offspring.

The data suggest that there may be similarities in maternal electroshock seizures and prenatal exposure to a BZ antagonist (but not an agonist). In addition, significant alterations in BZ binding appear to be associated with changes in seizure thresholds in developing animals.

139.7 DO GABA AGONISTS HAVE ANTICONVULSANT PROPERTIES ON THE PENICILLIN FOCUS IN RATS? R.G. Fariello and G.T. Golden. Medical Service, Neurology Section, VA Hospital and The University of Texas Health Science Center at San Antonio, Texas 78284.

It has been suggested that impaired GABA mediated inhibitory function plays an important role in the generation of penicillin induced epileptiform activity. We have, therefore, tested the action of i.v. administered direct GABA agonists on the activity of such an acute penicillin focus in rats. Under urethane anesthesia (1 g/kg), 200 and 400 IU of Na penicillin were injected in the sensorymotor cortex of two groups of rats. Progabide, 3APS, THIP were injected i.v. and their effect compared to the effect of Dilantin and of ethanol and DMSO which were used as solvents for Progabide. Total duration of spike activity, spike amplitude and spike frequency were compared between treated animals and control non-treated animals.

Immediate, transient spike suppression was noted only with Progabide (≥ 10 mg/kg, LD50 = 50 mg/kg i.v.). The average life of the focus was not altered by any of the compounds. Progabide decreased the spike frequency after the initial suppression effect in a non-statistically significant way. 3APS transiently decreased spike frequency in 2 out of 6 injections. THIP did not significantly affect penicillin spike but at doses greater than 5 mg/kg induced burst of spikes and polyspikes on the EEG. Dilantin increased spike frequency for about 30 minutes after injection.

These results differ from our own and other laboratory findings on the action of direct GABA agonists on penicillin spikes in cats, whereas the observed Dilantin potentiation is in agreement with previously reported studies in cats and rats. Since spike amplitude and duration were not affected in our experiments, we conclude that spike frequency, amplitude and duration of focal activities are not useful variables to be examined for prediction of anticonvulsant activity. Progabide showed the most consistent and persistent immediate transient suppression of spike activity. The relevance of this phenomena to the clinical anticonvulsant effect of Progabide and other direct GABA agonists is discussed.

139.8 MULTIPLE PHENYTOIN BINDING SITES: IDENTIFICATION AND CHARACTERIZATION IN BRAIN MEMBRANE. A.C. Bowling* and R.J. DeLorenzo, Dept. of Neurology, Yale Medical School, New Haven, CT 06510

Previous studies in our laboratory have demonstrated that micromolar concentrations of benzodiazepines inhibit the Ca^{2+}-calmodulin dependent protein kinase system in brain membrane (Science 213: 546, 1981). We have also shown that micromolar affinity benzodiazepine receptors exist in brain membrane which are distinct from the previously described nanomolar affinity benzodiazepine receptors (Science, in press). Benzodiazepine inhibition of protein kinase activity and benzodiazepine binding to the micromolar receptors correlate significantly with benzodiazepine inhibition of maximal electric shock-induced seizures. Displacement studies demonstrated that micromolar concentrations of phenytoin displace [^{3}H]-diazepam from the micromolar receptor. These findings suggest that the micromolar benzodiazepine receptor may represent a phenytoin receptor in brain membrane.

To examine the possible existence of specific phenytoin binding sites, receptor binding assays were performed on rat synaptic membrane utilizing a wide range of [^{3}H]-phenytoin concentrations (10^{-9} M to 10^{-3} M). Saturation studies demonstrated the existence of micromolar affinity phenytoin binding sites with a K_D of approximately 100 μM. Saturation studies in the nanomolar range revealed the presence of a second distinct class of specific phenytoin binding sites which exhibited a K_D of approximately 15 nM. It is possible that a third class of binding sites exists with a K_D of approximately 600 nM; however, the binding in this concentration range may represent low levels of binding to the micromolar affinity phenytoin binding sites. Displacement studies on the micromolar affinity phenytoin bindings sites demonstrated that micromolar concentrations of two other major anticonvulsant agents, diazepam and carbamazepine (Tegretol), displaced specifically-bound [^{3}H]-phenytoin. Conversely, we have shown that phenytoin displaces [^{3}H]-diazepam which is specifically bound to the micromolar benzodiazepine receptor.

These findings suggest that the micromolar phenytoin binding site is identical to the micromolar benzodiazepine receptor and thus this binding site may be a clinically-relevant phenytoin receptor which serves to mediate the anticonvulsant and neuronal stabilizing properties of phenytoin. These results are consistent with the hypothesis that specific phenytoin recpetors exist in brain membrane and that these binding sites may regulate some of the effects of several major anticonvulsants on neuronal tissue. The micromolar phenytoin receptor may represent a generalized anticonvulsant receptor that binds certain classes of anticonvulsant agents that are effective in inhibiting maximal electric shock-induced seizures.

139.9 EFFECTS OF α-METHYL-P-TYROSINE (α-MPT) ON PINEALECTOMY-INDUCED SEIZURES IN THE RAT. C. A. Stockmeier and D. E. Blask*. Dept. of Anatomy, University of Arizona, Tucson, AZ 85724.

Pinealectomy in gerbils or partially parathyroidectomized (PthX) rats causes seizures usually characterized by wild running followed by clonus, tonus and occasionally death. Norepinephrine (NE) is implicated in several animal seizure models. α-MPT lowers catecholamine levels in the gerbil telencephalon and makes the animal more susceptible to pinealectomy-induced seizures. To see whether inhibition of catecholamine synthesis exacerbates seizures in rats, we tested the effects of various doses of α-MPT on seizures in partially PthX rats that were later pinealectomized (PinX). Male Long-Evans rats (21 days old) were partially PthX. One week later, 4 groups of 9-10 PthX rats were PinX while a fifth group was sham-PinX. Groups of rats received an i.p. injection of either saline, 15, 25 or 35 mg of α-MPT 24 and 12 hours prior to pinealectomy and one more injection immediately after pinealectomy; the sham-PinX group received 35 mg of α-MPT according to the same schedule. Animals were observed continuously for eight hours after pinealectomy, and both the onset and type of each seizure were recorded. Seizures were recorded as follows: 1= wild run, 2=clonus + 1, 3=tonus + 1 + 2 and 4=death + 1 + 2 + 3. No convulsions were observed in the PthX, sham-PinX rats which received 35 mg of α-MPT. 90% of the control group of PthX rats that were PinX and injected with saline had convulsions and averaged 3.5 convulsions per rat during the 8-hour period. Averaged over the 8-hour period, none of the treatments with α-MPT changed the mean number of convulsions per convulsing rat, the number of rats that convulsed per number at risk or the latency to onset of any type of convulsion as compared with saline controls. However, significantly fewer convulsions occurred in rats treated with 15 or 35 mg of α-MPT as compared with the 25 mg treatment group. The 25 mg injections of α-MPT reduced by 49% the mean latency to onset of the first tonic convulsion when compared with saline controls. Conversely, latency to onset of the first tonic convulsion was restored to control values by 35 mg α-MPT. The analysis of data over time showed that during the second hour after pinealectomy, the 25 mg dose caused significantly more tonic convulsions per convulsing rat. Furthermore, a greater percentage of convulsions occurring during that hour were tonic when compared with saline controls. The high and low doses of α-MPT, thus, tended to diminish the seizures while the middle dose appeared to augment seizures early in the observation period. Therefore, these results do not unequivocally implicate the modification of catecholamine synthesis in convulsions induced by pinealectomy in PthX rats. Hence, the use of NE receptor agonists and antagonists may more clearly delineate the role of NE in this seizure model.

139.10 A COMMON ANTICONVULSANT DRUG EFFECT ON STANDARDIZED SLEEP EEG POWER SPECTRAL PROFILES IN THE RHESUS MONKEY. M. B. Sterman and R. A. Kovalesky*. V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Anatomy, Sch. of Med., UCLA, Los Angeles, CA 90024.

This study explored a new approach to the application of electroencephalogram (EEG) power spectral analysis in the search for an objective, quantitative evaluation of anticonvulsant drug effects on the primate central nervous system. Standardized EEG samples were drawn from the slow wave sleep state. Four rhesus monkeys were adapted to restraining chairs and to prolonged recording in an isolation cubicle. Chronically placed electrodes provided for the monitoring of sleep-waking states and of bipolar frontal, central and occipital EEG traces, bilaterally. Baseline spectral density data were drawn from standard successive and spaced recording nights, and from post-intramuscular saline injection recording nights. These were compared with identical data drawn 1.5-2.0 hours after a single, acute intramuscular administration of six compounds in counterbalanced order, including: diazepam, carbamazepine, dipropylacetic acid, diphenylhydantoin (DPH), phenobarbital and pentobarbital, the latter providing a non-anticonvulsant control. All dose values were in the range of low clinical norms and were confirmed by serum samples drawn immediately after EEG samples. Baseline measures showed stability across all non-drug test conditions, particularly those derived from sensorimotor cortex. Identical post-drug power spectral profiles indicated a unique and significant attenuation of 4-7 Hz activity bilaterally in central cortex for all test compounds except pentobarbital and DPH, when compared to baseline values. Since pentobarbital has no anticonvulsant action and DPH is ineffective in this regard when administered intramuscularly, these data indicate a common action of the remaining anticonvulsant drugs on central cortical EEG substrates. Other neurophysiological findings suggest that this may reflect an altered cortical response to intrinsic somatosensory thalamocortical afferent discharge in sleep, resulting from reduced neuronal excitability.

Supported by the Veterans Administration.

139.11 ELEVATION OF CSF FOLATE LEVELS FOLLOWING GRAND MAL SEIZURES IN UNTREATED PATIENTS. M.J.W. Brennan, J. Costa*, P. Ruff* and P. Sutej*. MRC Brain Metab. Res. Group, Univ. of the Witwatersrand and South African Inst. Med. Res., Johannesburg 2001, S. Africa

Folates exhibit both direct and facilitatory excitatory actions on neurons in the cat cerebral cortex (Davies, J. and Watkins, J.C., Biochem. Pharm., 22: 1667, 1973) and frog spinal cord (Loots, J.H. et al., J. Neural Transm., in press) and have potent convulsant properties in experimental animals. Olney and coworkers have demonstrated that injection of folates into the amygdala (Nature, 292: 165, 1981) or striatum of adult rats is capable of reproducing the sustained limbic seizures and disseminated pattern of brain damage characteristically associated with injection of kainic acid. This pattern conforms to that observed in autopsied brains of human epileptics. Since folates are present in central nerve endings and are released by depolarization in a partially Ca^{2+} dependent manner (Brennan, M.J.W. et al., Neurosci. Lett., 27:347, 1981) they might be involved in the initiation or spread of seizure activity in the brain. We have measured folate levels in the CSF of 8 male patients (28-60 yrs) who presented with a definitive history of grand mal seizures. Two presented in status epilepticus; none were receiving or had received antiepileptic drugs. Lumbar punctures performed 1-12 hours after seizures had ceased showed CSF folate levels (measured by Lactobacillus casei) of 20.5 ± 4.2 ng/ml (mean$\pm$S.E.M.) significantly raised compared to a group of age-matched controls (6.7 ± 1.3) Specimens were microscopically and bacteriologically normal. This finding coupled with the biochemical, anatomical and electrophysiological data already discussed supports the idea that folates released from nerve endings might interact with receptors (Ruck, A. et al., Nature, 287: 852, 1980) to play a role in the spread of seizures in the brain.

139.12 CHINABERRY JUICE: A NATURAL PRODUCT EXTRACT WITH ANTIEPILEPTIC PROPERTIES. B.B. Wannamaker, B.I. Diamond, D. Hammond*, J.R. Koonce*, W. Walter*. Department of Neurology, Medical University of South Carolina, Charleston, South Carolina 29425 and Department of Psychiatry, Medical College of Georgia, Augusta, Georgia 30912.

Chinaberry juice (CJ) was reported by an epileptic patient to effectively control her generalized tonic-clonic seizures and was used safely for longer than 14 years. A very interesting feature of her treatment was that CJ was consumed only once per week. CJ is obtained by boiling the berries of Melia azedarach (China-tree, Pride of India) which is indigenous to the southern United States. The pulp is then strained away and the remaining brew consumed.

CJ was administered to rats intraperitoneally (i.p.) 30 minutes prior to electroshock (140V, 0.05 msec, via corneal electrodes). The latency to seizures was increased and the duration of seizures was decreased. 73% of animals were protected against any seizure activity. CJ blocked i.p. and subcutaneous pentylenetetrazol (60 and 85mg/kg, respectively) induced seizures. The protective effect against pentylenetetrazol was seen also when animals consumed ad lib CJ for 2 days. Further, those animals which were protected by CJ were protected from daily pentylenetetrazol challenge for greater than 48 hours after CJ withdrawal. CJ did not block allylglycine (150mg/kg) induced seizures. Toxicity was seen with large i.p. doses of CJ; an estimate of the i.p. LD_{50} is four times the oral effective dose.

Numerous medicinal uses of extracts of Melia azedarach are purported. The literature reveals that extracted principles have effects against gastric ulcers, malaria and eczema; cardiovascular depressant effects and diuretic activity are described. Recently, experiments demonstrated antimitotic activity. Our patient experience and our preliminary studies indicate that chinaberry juice does contain substance(s) with effective antiepileptic activity which may have unique pharmacologic properties.

140.1 SOMATOSTATIN IN THE NEOSTRIATUM AND SUBSTANTIA NIGRA IN NORMAL HUMAN BRAIN AND IN HUNTINGTON'S DISEASE. P.E. Marshall*, G.D. Burd, and D.M.D. Landis (SPON: K.J. Sweadner). Dept. Neurology, Massachusetts General Hospital, Boston, MA. 02114

Huntington's Disease (HD) is accompanied by degeneration of most of the neurons in the caudate and putamen. In contrast to all other peptides thus far studied, the concentration of somatostatin is remarkably increased in the atrophic neostriatum in HD. We have used immunocytochemical techniques to compare the cellular distribution of somatostatin immunoreactivity in normal and HD brain.

Brain tissue obtained post-mortem from patients with HD and from age-matched patients without neurological abnormality was fixed by immersion in either 10% formalin, a modified Bouin's solution, or paraformaldehyde-lysine-periodate for 1 day to 6 weeks. Somatostatin-like-immunoreactivity (SLI) was demonstrated in 40μM Vibratome sections with unlabeled antibody techniques.

Neuronal perikarya containing SLI in normal neostriatum were 12-16μM in diameter, and gave rise to 2-4 dendritic processes. Stained cells were present throughout the caudate and putamen, sometimes in groups of 2-5. No clear staining was evident in the globus pallidus. In the substantia nigra (SN), varicose processes containing SLI were distributed sparsely among pigmented neuronal perikarya in the pars compacta and among their dendritic processes in the reticulata.

SLI was also present in medium-size neurons in the atrophic putamen and caudate in HD, and their dendritic arborizations appeared similar to those in normal brain. We have not detected a clear difference in the proportion of cells in the neostriatum containing SLI in normal brain as compared to HD brain. However, in HD brain there was a distinct population of varicose fibers containing SLI in the neostriatum. The fibers with SLI in the SN were identical to those in normal brain.

The increased concentration of SLI in the atrophic neostriatum in HD may be accompanied by an increase in the concentration of axon-like varicose fibers containing SLI. (Supported by NS 16367 and 00353)

140.2 EFFECTS OF KAINIC AND IBOTENIC ACID ON THE NEOSTRIATAL SOMATOSTATIN SYSTEM OF THE RAT. G.D. Burd, P.E. Marshall*, M.F. Beal*, D.M.D. Landis and J.B. Martin. Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Several neuropathological and neurochemical abnormalities which occur in Huntington's Disease (HD) are also observed in animals that have received striatal injections of kainic acid, including severe loss of small and intermediate size neurons, relative preservation of large neurons, and reductions in levels of several peptides and transmitters while dopamine levels are unaffected. Somatostatin levels are elevated in the neostriatum of HD brains. Immunocytochemical studies of HD brain do not demonstrate a disproportional survival of somatostatin-like-immunoreactive (SLI) neurons, but there does appear to be an increase in the concentration of varicose fibers with SLI. In the present study, we contrast lesions induced by kainic acid (KA) and ibotenic acid (IA) in rats with changes observed in HD.

Neurons containing SLI are present throughout the caudatoputamen, often in groups of 2-5 cells. Cell bodies are spherical (10μm diameter) with radial dendrites or bipolar (16 by 8μm). Often one of the dendrites is thicker than the others and branches into thinner processes; fine axonal processes occasionally originate from large dendrites. Many of the dendrites have varicose contours. At the ultrastructural level, the neurons are characterized by deeply invaginated nuclei and dendrites that appear to lack spines; they can be identified as medium aspiny neurons. A distinct population of fine beaded fibers containing SLI appears to originate from a region ventral to the anterior commissure and to distribute in the ventral and central caudatoputamen. Other fine, SLI fibers are present along the lateral ventricle.

Five to 7 days after injection of KA (5.9nmole) into the neostriatum, there is a well-circumscribed region in which all medium and small neurons degenerate. Four doses of KA (1.2-9.4nmole) yielded a graded response in SLI levels (no change to 57% decrease) as determined by radioimmunoassay (RIA). With IA (126nmole), fewer neurons degenerate and some SLI neurons are still present 6 days after the lesion; RIA data shows a decline of 13% compared to controls. Using a higher dose of IA (190nmole), there is a decline of 43%. We did not detect changes in SLI neurons or processes contralateral to the injections.

There does not appear to be selective survival of neurons containing SLI in rat neostriatum in KA- or IA-induced lesions, and we have not yet identified the appearance in the lesioned areas of varicose fibers like those seen in HD. (Supported by NS 16367 and NS 00353)

140.3 SYNAPTIC ORGANIZATION OF IMMUNOREACTIVE SOMATOSTATIN NEURONS IN THE RAT CAUDATE NUCLEUS. M. DiFiglia and N. Aronin. Dept. of Neurology, Mass. General Hospital, Boston, MA 02114 and Depts. of Medicine and Physiology, Univ. Mass. School of Medicine, Worcester, MA 01605.

Recent studies in our laboratories (DiFiglia and Aronin, J. Neurosci., in press) have used the peroxidase-antiperoxidase immunocytochemical method to examine the neuronal localization of immunoreactive somatostatin (iSS) in the rat caudate nucleus at the light and electron microscopic levels. Light microscopic results showed that iSS was contained within small to medium size neurons (9-20 μm in size). The dendrites of labeled cells were visible for long distances from the soma (up to 120 μm), were of different diameters, and exhibited smooth or irregular contours, varicosities, and fine beaded processes. At the ultrastructural level all iSS neurons had a deeply indented nucleus and a rich cytoplasm including well developed Golgi apparatus and small stacks of rough endoplasmic reticulum. The above features have been found in Golgi and Golgi-EM studies (see DiFiglia et al, J. Neurocytol., 9, 1980) to be associated with medium size aspiny neurons. This cell type has been observed to have a short axon.

In four rats examined for SS immunoreactivity, labeled fibers and terminals also were observed throughout the caudate nucleus. In an attempt to determine the morphologic features and synaptic connections involving these elements, we examined caudate neuropil in serial thin sections. Axonal fibers with SS immunoreactivity were mostly unmyelinated and of small diameter (0.08-0.2 μm). They gave rise to swellings along their course and to terminals which contained many pleomorphic vesicles and few large granular vesicles. Labeled boutons (N=51) were measured at the level of their synaptic contacts and showed a mean length of 0.57 μm (SD±0.22) and a width of 0.36 μm (SD±0.10). Of the total population sampled, 74.5 % (N=38) synapsed with the shafts of small or large dendrites and 23.5 % (N=12) contacted dendritic spines. Some of the spines were also postsynaptic to unlabeled axon terminals. Axosomatic synpases formed by iSS boutons were observed infrequently (2%; N=1). Serial sections also showed that some labeled terminals contacted more than one postsynaptic element. Most of the synaptic membrane densities appeared to be symmetric and had a mean overall length of 0.25 μm (SD±.09).

Taken together these results suggest that many of the iSS axons present in the rat caudate nucleus arise from medium size aspiny neurons and that they form intrinsic synaptic connections at least in part with caudate spiny neurons.

The somatostatin antiserum was generously provided by Drs. G. Nilaver and E. Lichtenstein. This research was supported by NIH grants NS 16367 (MD) and AM 01126-01 (NA).

140.4 RAPID ONSET OF SENSORIMOTOR DISORDER COINCIDES WITH ALTERATIONS IN NEOSTRIATAL DOPAMINE AND DOPAC LEVELS AFTER 6-HYDROXYDOPAMINE MICROINJECTION. C. A. Altar and J. F. Marshall. Dept. of Psychobiology, University of California, Irvine CA 92717.

The unilateral injection of 6-hydroxydopamine (6-OHDA) into the mesotelencephalic dopamine projection of rats results in a neglect of stimuli applied to the contralateral body surface (Marshall, J. F., Brain Res., 177:311, 1979). 6-OHDA neurotoxicity occurs within minutes *in vitro* (Heikkila, R. and Cohen, G., Mol. Pharm., 8: 241, 1972) and, if 6-OHDA inhibits impulse flow and dopamine release in nigrostriatal neurons, as do electrothermic lesions of this pathway (Walters, J.P. *et al*, J. Pharm. Exp. Ther., 186:630, 1973), sensory neglect and increases in neostriatal dopamine should appear almost immediately after 6-OHDA microinjection.

Freely moving male Fisher 344 rats received 8 μg injections of 6-OHDA via previously implanted cannulas whose tips terminated in the left ventral tegmental area. Rats were tested for their ability to orient to a 4-g force applied by a von Frey hair to 11 left or right body surfaces 30 min before and 10, 30, 50, 70, 90, and 110 min and 3 and 24 hr post-injection or were killed at 0, 10, 50, or 110 min post-injection. Dopamine, DOPAC, and HVA concentrations in the left and right neostriata of rats in the latter group were measured by HPLC with electrochemical detection. Somatosensory localization was measured in the former group as the percent of the total orientation score of the right body side compared to that of the left.

Somatosensory localization contralateral to the 6-OHDA injection declined to 70% at 10 min, 10% at 30 min, and remained at only 5% of ipsilateral localization scores at all subsequent times. The sensory neglect developed in a caudal to rostral manner.

Dopamine, DOPAC, or HVA concentrations were equal in the left and right striata at 0 and 10 min. Dopamine and DOPAC were elevated in the left striata compared to the right by 50% or 80% at 50 or 110 min, respectively, while HVA was increased only at 110 min post-injection. At no time did dopamine, DOPAC, or HVA levels in the right striata differ from control (0 min) values.

The rapid onset of both sensory neglect and increased dopamine content of the left striata is consistent with previous reports of decreased dopamine release from, and subsequent accumulation within, nigrostriatal nerve terminals after inhibition of impulse flow. 80% increases in neostriatal DOPAC have also been observed (di Giulio, A.M. *et al*, Eur. J. Pharm., 52:201, 1978) 60 min after γ-butyrolactone injection. Together, these results suggest that DOPAC may be produced intraneuronally *without* prior dopamine release and recapture by nigrostriatal terminals if impulse flow in this pathway has been blocked.

140.5 ASYMMETRIC DOPAMINE AND SEROTONIN METABOLISM IN THE NUCLEUS ACCUMBENS AND SUBSTANTIA NIGRA OF THE TRAINED CIRCLING RAT. Bryan K. Yamamoto* and Curt R. Freed (SPON: D.G. Whitlock). Depts. Med. and Pharm., U. Colo. Sch. Med., Denver, CO 80262

We have previously shown that the trained circling rat has increased dopamine metabolism in the caudate contralateral to the circling direction. Using this model we have investigated whether other brain areas known to be integrated with the caudate in the regulation of movement also show asymmetric neurotransmitter metabolism during circling. Both dopamine and serotonin (5HT) metabolism were studied in substantia nigra (SN) and nucleus accumbens (NA). Male Sprague Dawley rats were water deprived for 23 hrs and randomly chosen to be trained to turn either left or right for sucrose/water reward. After a one week training period, regional neurotransmitter activity was studied by sacrificing animals before, during, and after circling. SN and NA were bilaterally dissected and dopamine, 5HT and their metabolites were assayed by HPLC. Prior to circling, dopamine, DOPAC, 5HT, and 5HIAA concentrations were the same on both sides in all experiments. Results at all time points were calculated as the ratio of concentrations on the contralateral relative to the ipsilateral side. Elevated ratios indicate enhanced metabolism on the contralateral side. * Indicates significantly different from baseline ($p<0.05$). Values are Mean ± SE, n=6 at each time point.

N. ACC. CONTRALATERAL/IPSILATERAL CONCENTRATIONS RATIOS

	Baseline	Peak Circling	End Circling	2 hr Post-circling
Dopamine	1.01±.13	1.44±.05*	1.22±.11	1.11±.11
DOPAC	0.97±.13	1.90±.43*	1.46±.16*	1.51±.15*
5HT	0.86±.09	1.27±.33	1.34±.10	1.36±.22
5HIAA	1.04±.13	1.40±.28	1.26±.07	1.14±.19

SUB. NIGRA CONTRALATERAL/IPSILATERAL CONCENTRATION RATIOS

	Baseline	Peak Circling	End Circling	2 hr Post-circling
Dopamine	1.14±.23	0.94±.18	1.03±.06	1.40±.33
DOPAC	1.09±.15	0.73±.06	1.11±.13	1.11±.15
5HT	1.04±.08	1.23±.04*	1.37±.12*	1.47±.24*
5HIAA	1.04±.06	1.27±.09*	1.30±.08*	1.33±.17*

These data provide evidence for asymmetrically increased dopamine metabolism in the contralateral nucleus accumbens of the intact and undrugged rat without significant changes in 5HT or 5HIAA. Conversely, the substantia nigra contralateral to the trained circling direction showed enhanced 5HT metabolism but no changes in dopamine or DOPAC.

140.6 ASYMMETRIC CAUDATE TYROSINE HYDROXYLASE ACTIVITY IN THE TRAINED CIRCLING RAT. M.E. Morgan*, B. K. Yamamoto* and C. R. Freed Depts. Med. and Pharmacol., U. Colo. Sch Med. Denver, CO 80262.

We have previously shown enhanced accumulation, release, and catabolism of dopamine (DA) in rats trained to circle. The metabolism was increased in caudate contralateral to the turning direction. To further elucidate the mechanism of these changes, caudate tyrosine hydroxylase (TH) was studied. Male Sprague Dawley rats, 200-300g, were water deprived 23 hr and trained to circle 360° in a left or right-handed direction for a 10% sucrose reinforcement for 7 daily sessions. During the 8th session of circling behavior, animals were sacrificed at varying times, left and right caudate were removed and stored at -70° C. Each caudate was assayed within 24 hr for TH activity, DA and DOPAC concentrations using the methods of Nagatsu et. al. (Anal. Biochem. 9: 122-126, 1964) and Freed and Asmus (J. Neurochem. 32: 163-168, 1979), respectively. Enzyme activity (nmoles tyrosine oxidized/g/hr) was measured as a function of 6-methyltetrahydropterin (6-MPH4) concentrations from 0.04 to 1.0 mM. Lineweaver-Burk plots were used to analyze the kinetic parameters for TH. Although curvilinear relationships were seen with this technique, data were fit by linear regression analysis. Results are expressed as the ratio of enzyme activity or catechol concentration in caudate contralateral to the circling direction compared to ipsilateral caudate.

	Time (min) 0	20	70	280
Turns/min	0	11.1±.6	5.4±.4	1.2±.1
	Contralateral/Ipsilateral TH Ratio			
Km	1.09±.23	1.56±.29	1.33±.28	1.05±.11
Vmax	0.91±.15	2.02±.29*	1.32±.33	1.02±.09
	Contralateral/Ipsilateral Catechol Ratio			
DA	0.98±.03	1.65±.19*	1.18±.13	1.01±.09
DOPAC	0.91±.08	1.70±.20*	1.41±.22	1.04±.08

Values are Mean±SE, n=5; *Significantly different from 0 and 280 min, $p<0.05$.

The apparent Km for 6-MPH4 was not significantly changed with circling. However, the apparent Vmax was relatively increased in contralateral caudate when circling was most intense (20 min). DA and DOPAC contralateral concentration ratios were also significantly increased at this time point. These data are the first to show rapid asymmetric enhancement of TH activity during a specific motor task.

140.7 ELECTRICAL STIMULATION OF VARIOUS THALAMIC NUCLEI DIFFERENTIALLY AFFECTS THE RELEASE OF DOPAMINE IN THE TWO CAUDATE NUCLEI AND THE TWO SUBSTANTIAE NIGRAE, A. Cheramy*, M.F. Chesselet*, R. Romo*, V. Leviel* and J. Glowinski*.(SPON: S. Chorover), Groupe NB, College de France, Paris 75231, France.

The release of tritiated dopamine (^{3}H.DA) continuously synthetized from ^{3}H-tyrosine was measured in halothane anaesthetized cats by means of push-pull canulae implanted in both caudate nuclei (CN) and substantiae nigrae (SN), during and after unilateral electrical stimulations of various thalamic nuclei: 100uA, 1-2v, trains(100ms of 0.5msec shocks at 300Hz) delivered for 10min at 0.25 Hz. Stimulation of the ventralis lateralis and ventralis medialis nuclei (VL/VM) induced prolonged asymmetric changes of DA release in CN and SN (increase in the ipsilateral CN, decrease in contralateral CN, opposite responses in the SN) while the stimulation of the parafascicular(Pf) and adjacent centrum medianum (CM) nuclei induced a bilateral symmetric inhibition of DA release in both CN and both SN. More anterior stimulations of the CM induced an increase in DA release in the contralateral nigra. Stimulations of the centralis lateralis and paralamellar part of the medio-dorsal nucleus also induced only contralateral effects: inhibition of ^{3}H.DA release in the CN and SN. Stimulations of median nuclei had no effects. Thus the changes in DA release observed were highly dependent upon the exact site of stimulation and these results not only confirm the role of thalamic circuits in the control of DA transmission in CN and SN[1] but also suggest that different nuclei are involved in different patterns of changes in DA release such as those induced by different pharmacological manipulations of the SN or stimulation of other systems: both motor (VL/VM) and some non-specific sensory (CM/Pf) thalamic nuclei (or fibers passing through them) seem to be involved in the bilateral control of the two nigro-striatal dopaminergic pathways but while the stimulation of motor nuclei reproduced the asymmetric changes observed with stimulation of sensory afferences,[2] dentate nucleus of the cerebellum or changes in DA dendritic release,[3] the stimulation of the CM/Pf complex induced symmetric modifications as observed when GABA-ergic transmission is modified in one SN.[4]

1. Cheramy et al., Neurosci. 6 (1981) 2657-2668.
2. Leviel et al., Brain Res. 223 (1981) 257-272.
3. Cheramy et al., Nature 289 (1979) 537-542.
4. Leviel et al., Brain Res. 175 (1979) 259-270.

40.8 THE GABAERGIC PALLIDO-NIGRAL COMPLEX IN THE RAT DEMONSTRATED BY GLUTAMIC ACID DECARBOXYLASE IMMUNOCYTOCHEMISTRY. W.H. Oertel[+], E. Mugnaini[°], C. Nitsch*[¢], D.E. Schmechel*[#] and I.J. Kopin[@]. [+]Dept. Neurology, Technical Univ., Muenchen, FRG; [°]Dept. Biobehav. Sci., Univ. of Conn., Storrs, CT 06268 USA; [¢]Max-Planck Inst., Frankfurt, FRG; [#]Div. Neurology, Duke Univ., Durham, NC 27710 USA; and [@]Lab. Clin. Sci., NIMH, Bethesda, MD 20205 USA.

The globus pallidus (GP), the entopeduncular nucleus (EPN, the rodent homologue of the primate GP pars medialis), the substantia nigra pars reticulata (SNR), and the ventral pallidum are viewed as similar histologically, histochemically, and with respect to afferent innervation patterns. These nuclei are subsumed under the term "tier III" by H.J.W. Nauta (1979). The cytological similarities would assume larger significance if the neurons of these nuclei shared chemical properties. GABA has been proposed as transmitter of pallidofugal and nigrofugal projections (Penney & Young, 1981; Starr & Kilpatrick, 1981). We therefore investigated the localization of GABAergic neurons in rat GP, EPN and SNR by immunocytochemical methods using an antiserum to glutamic acid decarboxylase (GAD), the biosynthetic enzyme for GABA.

Normal rats and rats that had received a unilateral stereotaxic injection of colchicine (20 µg) near the area of interest 36 hr prior to sacrifice were perfused with buffered 4% formaldehyde/0.4% glutaraldehyde. Floating vibratome sections (25 µm) were processed with the PAP procedure for light and electron microscopy.

By light microscopic criteria near the site of colchicine injection, practically all neurons in GP and EPN and the large majority of neurons in SNR were GAD-immunoreactive. By contrast, only a few small neurons were GAD-positive in SN pars compacta. In GP, EPN and SNR, a large number of GAD-positive axon terminals were observed. These boutons contained pleomorphic synaptic vesicles and commonly formed symmetric synaptic junctions with dendrites and cell bodies, as already shown by Ribak et al. (1976, 1979). Postsynaptic elements were also GAD-immunoreactive in GP and EPN. In SNR numerous GAD-positive and fewer GAD-negative postsynaptic dendrites and cell bodies were observed.

We confirm the predominant GABAergic nature of boutons in GP, EPN and SNR. Furthermore, the data indicate that GABAergic neurons are the predominant cell type in the rat pallido-nigral complex and support the notion that "tier III" nuclei are inhibitory.

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140.9 PROJECTION NEURONS OF THE PARS RETICULATA OF THE SUBSTANTIA NIGRA IN MONKEY AND CAT: MULTIPLE RETROGRADE CELL-LABELING WITH FLUORESCENT DYES. Robert M. Beckstead, Dept. of Anatomy, Univ. of Virginia Schl. of Med., Charlottesville, VA 22908

The pars reticulata of the substantia nigra (SNR) is increasingly appreciated as a major outroad for the activity of the corpus striatum. The three major targets of SNR projections are the thalamus (VA-VL complex, MD and intralaminar nuclei), the intermediate gray layer of the superior colliculus, and the caudal midbrain reticular formation including the pedunculopontine nucleus. Our lab has mapped the intranigral distribution of SNR cells that project to each of these structures in monkeys using the HRP method (Beckstead & Frankfurter, Neuroscience, 1982). In order to gain some impressions about the degree to which individual SNR cells send collateral branches to more than one of the three major targets, we have injected two or all three of them with fluorescent dyes (Evans Blue, Propidium Iodide, Granular Blue, Fast Blue, Nuclear Yellow) in six squirrel monkeys and eight cats. The best results were obtained in the monkey brain with injections of Evans Blue in the thalamus, Granular Blue in the colliculus and Nuclear Yellow in the reticular formation. The distributions of neurons in SNR that contained only one dye are consistent with our earlier HRP data such that nigrothalamic and nigroreticular cells are numerous and widely scattered throughout all zones of SNR. Nigrotectal cells, on the other hand, are restricted to rostral and lateral zones of SNR. Double labeling of cells indicates that many of the more caudally located nigroreticular cells also send a collateral to the thalamus as do a smaller number of very rostrally located nigrotectal cells. The highest frequency of double-labeling occurs in laterally located cells that project to both the colliculus and caudal midbrain reticular formation. Less than ten cells in any given case could be confidently identified as containing all three dyes and these cells are all located laterally in the rostral half of SNR. In the cat, thalamic injection of Granular Blue and tectal injection of Nuclear Yellow indicate that most nigrotectal cells are located in the middle of the mediolateral expanse of SNR in its rostral one-half or so. Nigrothalamic cells flank the nigrotectals both medially and laterally. Where these groups border one another, several cells contain both dyes indicating that about 10 to 20% of SNR neurons in the cat send collaterals to both the thalamus and colliculus. In conclusion, our results indicate that several SNR projection neurons send collateral branches to two or three of the major targets, but the majority of SNR cells appear to project to only the thalamus, colliculus or reticular formation.

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140.10 NIGRAL SYNAPTIC ACTIONS ON TECTAL NEURONS. AN INTRACELLULAR HRP STUDY. A.B. Karabelas* and A.K. Moschovakis* (SPON. A.Paldino). Dept. Neuroscience, Albert Einstein Coll.Med., Bx., NY 10461

Intracellular recording was employed to determine the pattern of evoked PSPs in tectal neurons during nigral stimulation in barbiturate anesthetized intact cats. Corticotectal fibers traversing the substantia nigra are activated during nigral stimulation. Therefore, synaptic effects of nigral stimulation were compared to effects of pericruciate cortex and cerebral peduncle stimulation. Postsynaptic tectal neurons were intracellularly injected with HRP.

Nigral stimulation elicited monosynaptic IPSPs in tectal neurons with latencies of 1.5-1.83msec (mean latency: 1.65msec). Cerebral peduncle and cortical stimulation had no synaptic effect on these neurons. Most of the HRP labeled tectal neurons were located in the stratum griseum intermedium (SGI) and fewer in the stratum griseum profundum (SGP). They were small to medium size(300-1000μ^2) neurons with various soma shapes (fusiform, granular, conical, ovoid or stellate) and isodendritic or leptodendritic branching pattern. Their axons (1-2.5μ in diameter) consistently emitted collaterals. Intrinsic varicose collaterals were confined in the deeper tectal layers. Extrinsic collaterals were observed to terminate in the central gray and the midbrain reticular formation (MRF). The pattern of traced axonal trajectories suggests that tectal neurons receiving monosynaptic inhibitory nigral input give rise to either crossed or uncrossed descending projections. Moreover, their axon may branch to the opposite superior colliculus.

Nigral and cerebral peduncle stimulation evoked monosynaptic EPSPs in another group of tectal neurons with latencies of 0.65-1.65msec (mean latency: 1.04msec) and 0.8-1.35msec (mean latency: 1.16msec) respectively. Cortical stimulation, when effective, also elicited EPSPs. These neurons were located in both the SGI and SGP. They were large (950-3500μ^2) usually triangular cells with spherical or ovoidal dendritic fields. Their axons (4-7μ in diameter) followed crossed descending trajectories. Only one axon emitted a bifurcating collateral terminating in the MRF and the interstitial n. of Cajal.

IPSPs preceded by a brief EPSP were recorded from a third group of tectal neurons during nigral stimulation. Latencies of these EPSPs were similar to the latencies of pure evoked EPSPs. These neurons were located predominantly in the SGI. They were medium size to large (600-1700μ^2)cells with multipolar somas and radiating or occasionally vertical dendritic fields. Their axons (2-4μ in diameter) followed either crossed or uncrossed descending trajectories and rarely emitted intrinsic and/or extrinsic collaterals.

The morphophysiological data obtained in this study establish the existence of inhibitory monosynaptic nigral input to tectal projection neurons. It is also suggested that excitatory potentials are due to concomitant activation of corticotectal fibers. (Supported by NIH NS-07512).

140.11 FUNCTIONAL REGULATION OF GABA TURNOVER IN DEEP AND SUPERFICIAL LAYERS OF SUPERIOR COLLICULUS: INFLUENCE OF DOPAMINERGIC TRANSMISSION. M. R. Melis* and K. Gale. Dept. of Pharmacology, Georgetown Univ. Sch. of Med. and Dent., Washington, DC 20007.

The nigrotectal pathway represents an important descending outflow from the basal ganglia. Recent evidence has indicated that this pathway includes an inhibitory GABAergic component, originating in substantia nigra pars reticulata and terminating in the deep layers of superior colliculus (SC). In the present studies we evaluated the turnover rate of GABA in the deep and superficial layers of the SC and determined whether this index of GABAergic function was sensitive to manipulations of dopaminergic (DA) transmission. To estimate GABA turnover rates, we measured the initial rate of accumulation of GABA (over 90 min) following local microinjection of the irreversible inhibitor of GABA transaminase, gamma-vinyl-GABA (Casu and Gale, Life Sci 29:681, 1981), applied unilaterally to the deep and superficial layers of SC via indwelling cannulae. The rate of accumulation of GABA was 1.0 and 1.2 nmol/mg prot/min, respectively, for the deep and superficial layers of SC. Stimulation of DA transmission by treatment with cocaine (30mg/kg ip at 30min intervals), amphetamine (10mg/kg sc) or apomorphine (3mg/kg sc at 30min intervals) significantly decreased the rate of accumulation of GABA in the SC; this effect was consistently more pronounced in the deep layers than in the superficial region:

Rate of Accumulation of GABA: Percent of Control

	Saline	Apomorphine	Amphetamine	Cocaine
Superficial SC	100 ± 6	80 ± 5	85 ± 4	85 ± 4
Deep SC	100 ± 6	70 ± 6	60 ± 5	55 ± 4

The dopamine-receptor antagonist, haloperidol (2mg/kg ip), did not affect the rate of accumulation of GABA in SC when given alone, but prevented the depression in GABA turnover in SC produced by the DA agonists. This suggests that tonic DA transmission is not necessary for the maintenance of the basal turnover rate of GABA in SC, but that augmentation of DA transmission can suppress the utilization of GABA in this nucleus.

Studies performed in rats in which one eye was removed indicated that the influence of apomorphine on GABA turnover in the superficial layer of SC may be dependent on activity in visual pathways. In contrast, it appears that the influence of DA agonists on GABA turnover in the deep layers of SC is dependent upon neural connections between the striatum and tectum via substantia nigra. We propose that stimulation of DA receptors in striatum causes a disfacilitation of nigrotectal GABAergic projections which in turn produces a net disinhibition of neurons in SC.

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140.12 FUNCTIONAL REGULATION OF GABA TURNOVER IN THE MESENCEPHALIC RETICULAR FORMATION: INFLUENCE OF DOPAMINERGIC TRANSMISSION. Judith A. Childs* and Karen Gale (SPON: J. Neale). Dept. of Pharmacology, Georgetown Univ. Sch. of Med. & Dent., Washington, D.C.

The nigrotegmental pathway is one of four major efferent pathways from substantia nigra (SN). Our previous evidence (Soc.Neurosci.Abstr.,64.21(1981)) suggests that this projection is, at least in part, GABAergic. The purpose of the present studies was to determine whether dopaminergic drugs influence the turnover rate of GABA in the terminal region of this projection. The rate of accumulation of GABA was measured in rats following microinjection of the GABA transaminase inhibitor, gamma vinyl-GABA (GVG, 20 ug/ul), placed unilaterally into regions of reticular formation (RF) surrounding and including the pedunculopontine nucleus (PPN). Apomorphine (3 mg/kg sc), a dopamine agonist, was injected at 1/2-hour intervals. The rate of accumulation of GABA at timepoints during the first 90 min following GVG administration was used as an index of GABA turnover. Under these conditions, apomorphine treatment caused an enhanced rate of GABA turnover in the areas containing nigrotegmental terminations. These results are in direct contrast to those obtained in the terminal regions of the nigrotectal pathways (Melis and Gale, this volume). Moreover, in apomorphine-treated rats, GABA agonists placed in the vicinity of the PPN caused contraversive circling, in contrast to the ipsiversive circling observed with placement of GABA agonists into the tectal region. Our results suggest that the descending outflows from the basal ganglia may be differentially influenced by dopamine receptor stimulation. A possible role of striatonigral projections in the mediation of these effects is being investigated.

Supported by HHS grants DA-02206 and MH-32359.

141.1 MORPHINE SELECTIVELY REDUCES C FIBER PAIN IN HUMANS. B.Y. Cooper, C.J. Vierck, Jr. and O. Franzen*. Dept of Neuroscience, College of Medicine University of Florida, Gainesville, FL, 32610.

In previous experiments with monkeys, we have confirmed the difficulties that have been encountered in rodents in achieving the expected hypalgesic effects of morphine at low doses. While reflexive measures give approximate estimates of pain threshold, they do not reliably reflect the intensity of suprathreshold stimulation and are relatively insensitive to morphine. The frequency of intertrial behaviors reflects highly individualistic response patterns that are not specific to pain paradigms but are highly sensitive to morphine. Operant escape latencies are highly correlated with pain in most animals but are susceptible also to the non-specific effects of morphine. Of the pain correlates we have examined, only operant response force accurately quantifies pain, is sensitive to morphine, and is resistant to non-specific effects (Cooper and Vierck, Soc. Neurosc. Abst., 6: 430, 1980; Vierck et al., Progress in Psychobiology and Physiological Psychology, 1982). Yet the doses necessary to produce hypalgesia in monkeys by this measure (1-2 mg/kg; M. speciosa) are 10 to 20 times higher than those used therapeutically in humans. While the differences may be due to disimilar opiate receptor distribuiton or concentration in the two species, the differences are likely to be due to other factors as well.

Morphine preferentially reduces C fiber input to spinal cord cells of the rat at relatively low doses (.5 mg/kg; Jurna and Heinz, Brain Res., 171: 573, 1979). Thus, it is possible that the reduction of pain originating in C fibers is the most important clinical effect of systemic morphine. In the present study, human subjects were trained to attend selectively to either the fast (A-delta) or slow (C fiber) pain sensations following a strong electrical stimulus. A powerful reduction of C fiber pain by morphine was found. But there was no evidence of reductions in A-delta pain. It is likely that A-delta pain is reduced only at higher systemic doses, such as those commonly used in laboratory animal experiments. Experiments using stimuli which elicit primarily A-delta pain may not yield results that are relevant to the common clinical use of morphine -- that is, administration of a low dose by a systemic route. Reduction of A-delta pain could result from epidural or intraparenchymal administration of opiates, where the local concentrations of morphine are much higher. The study of mechanisms of systemic morphine analgesia should proceed with the development of humane models of C fiber pain in animals. (This research sponsored by PHS grant NS 07261.)

141.2 PITUITARY-ADRENAL AXIS AFFECTS SENSITIVITY TO MORPHINE ANALGESIA. S.J. Slater*, J.W. Lewis, G.W. Terman*, and J.C. Liebeskind, Dept. of Psychology, University of California, Los Angeles, CA. 90024. (SPON: A.N. Taylor)

Repeated exposure to painful/stressful stimulation causes a dramatic sensitization to morphine analgesia. This enhanced responsiveness can be produced by a variety of stressors (e.g., hotplate, footshock), is critically dependent on the number and intensity of stress exposures, is specific to the environment in which the stimulation occurred, persists several days following the last stress experience, and is dependent upon changes within the brain (Sherman et al., 1981; Lewis et al., 1982). Since pituitary and adrenal hormones (e.g., ACTH, corticosterone) are thought to influence morphine analgesia and are released by stress, the following investigations were undertaken to describe the role of this endocrine system in stress-induced potentiation of morphine analgesia.

Groups of male rats were brought into the testing environment daily for 7 days where they received either footshock stress (2.5 mA, 3 min), an injection of the synthethic glucocorticoid dexamethasone (400 µg/kg, s.c.), or no stress and served as controls. On Day 8, animals were returned to the test room and their analgesic response to morphine (5 mg/kg, s.c.) was assessed using the hot-plate test. In other rats receiving either 7 days of footshock or no stress, blood samples were collected in the test room on Day 8 and serum corticosterone levels were determined by RIA.

Animals receiving either footshock or dexamethasone demonstrated enhanced morphine analgesia ($p < .01$, compared to controls). Basal pain response latencies were also increased in these rats ($p < .05$) suggesting the development of a conditioned analgesic response. Finally, compared to controls, rats exposed to chronic footshock displayed significantly elevated serum corticosterone levels on Day 8 ($p < .05$).

Thus, stress-induced potentiation of morphine analgesia appears to be associated with alterations in the pituitary-adrenocortical axis. Those animals most sensitive to morphine had higher serum corticosterone and, presumably higher ACTH levels; and the enhancing effects of chronic stress were mimicked by chronic steroid administration. These data suggest that repeated exposure to painful/stressful stimuli may come to elicit a conditioned release of pituitary-adrenal hormones that serve to facilitate morphine analgesia. At this time, however, we are unable to discern which hormone, ACTH or corticosterone, is responsible for this effect. (Supported by NIH grant NS07628.)

141.3 CONTINUOUS INTRATHECAL INFUSION OF MORPHINE OR ARTIFICIAL CSF ON THE DORSUM OF THE SPINAL CORD VIA AN OSMOTIC MINIPUMP REDUCES AUTOTOMY IN NERVE SECTIONED RATS. Z. Wiesenfeld-Hallin, Department of Clinical Neurophysiology, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

Rats often respond to limb nerve section by performing autotomy in the deafferented region. This behavior may be an experimental model to study certain aspects of chronically painful conditions that can arise after peripheral nerve lesion or amputation in man. There is evidence that abnormal properties of the nerve sprouts in the neuroma that is formed subsequent to nerve section underly autotomy behavior. Factors such as stress and genetic variations also have important effects on autotomy (Wiesenfeld and Hallin, Physiol. Beh., 1981, 27).

In this study the effects of intrathecally administered analgesics on autotomy were examined. An intrathecal catheter extending to the lumbar enlargement (LE) was inserted through the cisterna magna (Yaksh and Rudy, Physiol. Beh., 1976, 17) in male Sprague-Dawley rats. After one week of recovery the sciatic nerve was sectioned and ligated unilaterally. An osmotic minipump (Alza, model 2002) delivering a nominal dose of 240 µg/day morphine HCl or an equivalent volume (12 µl/day) of artificial CSF was connected to the catheter. Autotomy was scored daily for the 14 days that the pump was delivering the solution. After completion of the experiments the rats were perfused and the spinal cord was examined to determine the location of the catheter tip.

Autotomy was significantly reduced during the 14 day period of infusion of morphine HCl and artificial CSF in rats in which the catheter tip was located on the dorsum of the LE in comparison to rats where the catheter tip was under the dorsal roots or rostral or caudal to the LE. Autotomy in rats infused intrathecally where the catheter tip was properly localized was also significantly reduced in comparison to rats that underwent sciatic nerve section but did not receive drugs intrathecally.

The observed reduction in autotomy in rats infused with analgesic substances on the LE further enhances the relevance of this animal model for experimental studies of chronic pain. The results support previous observations (Wiesenfeld and Gustafsson, Brain Res., in press) that both morphine HCl and artificial CSF have analgesic effects by this method of administration. The catheter tip must be accurately positioned to obtain analgesia because the small volumes infused are highly localized. Continuous intrathecal infusion of drugs may be a useful technique in experimental studies of chronic pain conditions.

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141.4 DEPLETION OF BRAIN SEROTONIN DOES NOT AFFECT AUTOANALGESIA. W. T. Chance and K. Minnema*. Department of Surgery, University of Cincinnati Medical Center, Cincinnati, OH 45267.

Recent reports have suggested that stress-induced analgesia may be mediated by at least two separate neurochemical systems. Thus, analgesia induced by prolonged stress appears to be opioid in nature, while the analgesic response to acute stress is not attenuated by classical opiate antagonists. We have previously reported that analgesia induced by acute stress may be classically conditioned to appropriate environmental stimuli. This conditioned analgesia is also resistant to blockade by naloxone and naltrexone, suggesting nonopioid mediation. Since much of the pain and analgesia literature has focused upon CNS serotonergic (5-HT) systems, we investigated the effects of depletion of brain 5-HT with para-chloroamphetamine (PCA) upon analgesia elicited by footshock and classical conditioning procedures. Analgesia was assessed using a radiant-heat tail-flick procedure, employing basal responses of approximately 3 sec and a cut-off criterion of 8 sec. In the first experiment PCA (10 mg/kg) was administered (ip) to 14 female S-D rats, while 15 additional rats were injected with saline. One week later, footshock (1 ma, 15 sec) was administered to half of each group of rats, while the remaining rats received control manipulations. Tail-flick latencies were again determined immediately after the footshock. Across the next 7 days tail-flick latencies continued to be assessed in PCA and control rats prior to the administration of footshock to determine classically conditioned analgesia. All rats were immediately sacrificed following the last tail-flick test, with their brains being removed and frozen in liquid N_2. In the second experiment analgesic testing was begun on male, S-D rats 10 days after the administration of PCA (10 mg/kg, ip, n = 21) or saline (n = 18), with all rats being sacrificed 1 week thereafter. The brains of these rats were separated into forebrain and brainstem sections by a vertical cut at the level of the superior colliculus. In these experiments CNS monoamine levels were determined primarily by HPLC procedures following homogenization in formic acid-acetone. Although PCA reduced whole brain 5-HT by 60% and 5-hydroxyindoleacetic acid (5-HIAA) by 68%, no significant effects were observed on analgesia elicited by footshock or within the classical conditioning paradigm. Whole brain levels of norepinephrine (NE) or dopamine (DA) were not affected by either PCA or the conditioning treatments. In the second experiment PCA again had no effect on either analgesic response, while brain stem 5-HT and 5-HIAA were reduced by 50%. No effects of any treatments were observed on brain stem catecholamines or metabolites (homovanillic acid, dihydroxyphenylacetic acid, 3-methoxytyramine). Thus, these data de-emphasize a role of CNS 5-HT in the mediation of autoanalgesic responses.

141.5 HYPERALGESIA AND ANALGESIA INDUCED BY THE SAME STRESSOR. Dennis D. Kelly, Pierre A. Lemaire* and Donald S. Leitner
New York State Psychiatric Institute and Dept. of Psychiatry, Columbia University, New York, NY 10032

When an animal's responsivity to pain is tested following exposure to a stressor, the method selected to measure pain is a major determinant of the results obtained and the conclusions drawn. For example, the time course of analgesia induced by a brief, forced cold-water swim ranges from minutes to hours depending upon whether pain thresholds are measured by operant liminal escape, reflex flinch-jump (FJ) or reflex tail-flick (TF) procedures. In the present pair of experiments, we observed that a single stressor, inescapable footshock, produced both analgesia and hyperalgesia in the same subject depending upon how pain thresholds were measured. Moreover, both effects were enhanced by hypophysectomy.

In Exp I five male albino rats were exposed to inescapable footshock stress (IFS) consisting of 300-msec shocks of 3.5 ma repeated at 2-sec intervals for 5 mins, the same parameters reported by Millan et al (Pain, 1980, 8, 343-353) to induce tail-flick analgesia. Each rat was exposed four times to IFS and combined FJ/TF testing occurred either 0, 15, 30 or 60 mins later. Three no-stress baseline FJ/TF test days separated the IFS sessions. The sequence of exposure to the IFS conditions was counterbalanced across rats in an incomplete Latin Square design. FJ thresholds were decreased for 30 mins following IFS, while TF thresholds were increased. These paired results demonstrate that the method employed to assess pain thresholds can determine the direction of threshold shifts induced by stress.

In Exp II 14 male hypophysectomized (HYPOX) and 14 sham operated adult rats were exposed to IFS followed by FJ/TF testing 10 mins later. The animals were stressed twice during the 2nd postoperative week and twice again in the 14th week following regeneration of the neurohypophysis (and recovery of water balance). Following sacrifice in the 15th week the adrenals and testes of all rats were dissected and weighed, and any tissue remnants in the sella turcica were fixed and sectioned to determine pituitary status. The results were as follows: Normal FJ and TF thresholds did not differ between HYPOX and sham rats in the 2nd week, nor in the 14th week when adjusted for divergence in body weight. However, when stressed, HYPOX rats displayed both potentiated TF analgesia and potentiated FJ hyperalgesia. The greater responsivity to FJ shocks would suggest that the potentiation of TF analgesia induced by IFS in HYPOX rats was not due to a motor deficit.

(Supported by PHS Grant MH 15174)

141.6 REDUCTION OF MORPHINE ANALGESIA AND BASAL NOCICEPTION FOLLOWING CENTRAL PARACHLOROPHENYLALANINE TREATMENT. J.H. Kordower, R.J. Bodnar, A. Reches, D.A. Simone*, M.M. Wallace and S. Fahn. Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367 and Dept. of Neurology, Columbia Univ. Col. of Physicians and Surgeons, New York, NY 10032.

Systemic administration of the tryptophan hydroxylase inhibitor, parachlorophenylalanine (PCPA) increases an animal's sensitivity to noxious electric shock and decreases the analgesic effects of morphine. The time course of such effects appears to parallel PCPA-induced serotonin depletion. However, this route of administration also elicits other non-specific peripheral effects including lethargy, illness and neglect of the ventral surface of the body. To determine whether PCPA-induced alterations in pain thresholds were due to central, rather than peripheral, mechanisms, four groups of eight rats each received an intracerebroventricular injection of a 1 mg dose of PCPA, a 3 mg dose of PCPA, normal saline or an equimolar hypertonic saline solution respectively. Flinch-jump thresholds were determined prior to injection, 0.5 h following injection and then at daily intervals for 1 week following injection. While animals treated with either of the vehicle solutions or the lower PCPA dose failed to display shock threshold changes, the 3 mg PCPA dose significantly decreased jump thresholds at 0.5, 48 and 120 h following injection.

To assess whether central PCPA administration reduced morphine analgesia as a function of pretreatment interval, groups of eight rats each received an intracerebroventricular injection of a 3 mg dose of PCPA at either 0.5, 24, 48 or 72 h prior to administration of a 5 mg/kg dose of morphine. A fifth group received a normal saline injection 0.5 h prior to the opiate. PCPA effects upon morphine analgesia varied as a function of pretreatment interval in that morphine analgesia was unaffected 0.5 h after PCPA, diminished after 24 h elapsed, eliminated at 48 h after PCPA and recovered at 72 h after PCPA. Vehicle-treated animals displayed normal morphine analgesia. These data will be discussed in terms of the relative effectiveness of central PCPA injections to deplete brain serotonin and norepinephrine as a function of pretreatment interval. Supported by NIH GRSG 5-S05-RR-07064.

141.7 THE ROLES OF CONTROLLABILITY AND STRESS DURATION IN ANALGESIC RESPONSES OF MSG-TREATED RATS. J. Toole*, J. Williams* and K. King. Psychology Dept., Kenyon College, Gambier, Ohio 43022.

Recent research has shown that stress-induced analgesia (SIA) in laboratory rats probably comes in at least two forms. Stressors of short duration (such as a cold-water-swim (CWS), continuous shock, or injections of 2-deoxyglucose (2-DG) produce an analgesia which is temporally bound to the stimulus and which is not naloxone-reversible. Long-lasting stressors such as an 90-minute train of inescapable shocks lead to activation of a naloxone-reversible analgesic mechanism. Surprisingly, these shocks do not produce SIA if the animal is allowed to escape the shocks. This mechanism, unlike the other, can be reinstated 24 hours after the noxious stimulus by exposure to a few "priming" shocks.

Neonatal treatment with monosodium L-glutamate (MSG) produces selective damage in basal hypothalamic areas which are involved in B-endorphin and ACTH release. Preliminary data indicate that MSG-animals do not show the normal analgesic response to short-term stressors like CWS and 2-DG. No information is available on the response, if any, of these animals to the long-term form of stressor. It was the purpose of these two experiments to investigate interactions between MSG-damage, the two forms of SIA and the factor of shock-controllability.

In the first experiment 13 animals which had been treated with MSG and 21 normal littermates (7 of whom were randomly selected for injection with naloxone) were all found to show SIA after a 3-min swim in 2°C water. Injection with 2-DG, however, failed to produce SIA. For experiment 2, the MSG, normal, and naloxone subjects were randomly divided into two groups which received either 80 escapable or 80 inescapable tail-shocks. While most groups showed SIA, the variable of escapability did not produce consistent results. Not only did all the inescape groups (including naloxone) show SIA, but the normal-escape group did as well; only the MSG-escape group failed to show SIA. Strangely, this group, as well as all the others except the naloxone group, did show an analgesic response when given five priming shocks 24 hours later. The inconsistency of this pattern indicates that inability to control shock may not be requisite for the induction of SIA of the long-term, reinstatable type. Two consistent patterns were seen with regard to the MSG animals: 1) MSG animals have significantly higher baseline pain thresholds; 2) MSG rats show both forms of SIA in a manner virtually indistinguishable for the normal controls. These two findings indicate the preservation of basic analgesia mechanisms concomitant with a resetting of their sensitivity, and are discussed in light of the probable circuits damaged by MSG.

141.8 TAIL PINCH HYPERALGESIA: DOPAMINE AND OPIOID MODULATION. D.A. Simone*, R.J. Bodnar, A. Reches, J.H. Kordower and S. Fahn. (SPON: R.J. Bodnar). Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367 and Dept. of Neurology, Columbia Univ. Col. of Physicians and Surgeons, New York, NY 10032.

Mild, non-noxious, oscillating pinches to a rat's tail elicit hyperphagia and hyperalgesia. Moreover, tail pinch (TP) reduces the analgesic responses following morphine and cold-water swims. Since TP hyperphagia appears to be mediated in part by dopaminergic and opioid mechanisms, based upon observed reductions following 6-hydroxydopamine (6-OHDA) and naloxone respectively, animals receiving these manipulations as well as rats receiving chronic morphine were assessed for TP hyperalgesia. In Experiment 1, groups of rats received either intracerebroventricular (icv) 6-OHDA (250 ug) and systemic desimiprimine (DMI: 40 mg/kg), icv vehicle and systemic DMI, or icv and systemic vehicle. Jump thresholds were measured in the presence (TP) and absence (NO TP) of TP prior to and up to four weeks after injection. 6-OHDA rats continued to display TP hyperalgesia despite increased jump thresholds in both TP and NO TP conditions. In contrast, DMI alone produced a transient loss of TP hyperalgesia which was due to increased TP jump thresholds. 6-OHDA increased the amount of time eating, licking and gnawing the food pellets relative to the DMI and vehicle groups.

In Experiment 2, nine rats received intraperitoneal injections of naloxone at doses of 0, 0.1, 0.5, 1, 5 and 10 mg/kg in counterbalanced fashion 5 min before jump threshold determinations following TP and NO TP conditions. Naloxone altered TP hyperalgesia as a function of dose in that the 0.1 and 0.5 mg/kg doses eliminated the hyperalgesia by lowering NO TP jump thresholds. While the 1 and 5 mg/kg doses failed to alter TP hyperalgesia, the 10 mg/kg dose eliminated this effect by increasing TP jump thresholds. Furthermore, while the amount of time eating chewing and gnawing decreased at high naloxone doses, this effect failed to differ significantly from vehicle values.

In Experiment 3, rats received 14 daily subcutaneous injections of either morphine (10 mg/kg, n=9) or vehicle (n=6) and both TP and NO TP jump thresholds were determined prior to and 1 and 14 days following the injection sequence. While vehicle rats displayed TP hyperalgesia, chronic morphine eliminated TP hyperalgesia 1 day after the injection sequence by lowering NO TP jump thresholds. Chronic morphine failed to alter TP hyperphagia. These data suggest that both dopaminergic and opioids modulate TP hyperalgesia, but the modulation involves complex and dissociable components. Whereas 6-OHDA alters both TP and NO TP thresholds, various naloxone doses and chronic morphine differentially alter one, but not the other. (GRSG 5-S05-RR-07064)

141.9 EVIDENCE FOR TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION-INDUCED ANALGESIA IN SEVERAL PAIN MODELS IN THE RAT. J.A. Heltzel and K.M. Westphall* (SPON. H. Bantli) Biosciences Research Lab., 3M, St. Paul, MN 55144.

Various forms of electrostimulation have been shown to be efficacious for the relief or reduction of pain in the laboratory animal as well as the clinical setting. However, transcutaneous electrical nerve stimulation (TENS) has not been well-studied in the classical laboratory animal models. Woolf et al.(1977,1980) have reported that percutaneous stimulation increases tail flick latency in the heated bath/tail immersion paradigm, commenting that similar findings were obtained with TENS. We confirm the latter finding and report TENS-induced analgesia in two other classical models (chemical writhing and radiant heat/tail flick) in the rat.

In each model TENS was delivered through self-adhering, reusable electrodes (3M,No. 6225) placed bilaterally at the base of the tail for the tail flick tests and across the lower thoracic region for the irritant-induced writhing. A balanced current (∿10mAmp, 100 Hz, 200 μsec width) was applied for 30 minutes.

The latency of the flexor withdrawal reflex was measured by immersing the tip of the tail in a 49°C bath. Baseline latencies were recorded, stimulation was applied, and post-TENS latencies were measured so that each animal served as its own control. Unstimulated rats were included in blind studies as a control for bias. Greater than 70% of the TENS group displayed significantly longer latencies, being 30-60% of baseline values ($p < .005$); these increases persisted for at least one hour post-stimulation.

In a similar design noxious radiant heat was delivered to the tip of the tail by a focused beam with an automated system for the timing of withdrawal latencies. The difference in latency before and after the application of TENS was compared to the latency difference recorded for restrained, unstimulated controls. Under blind conditions, post-TENS latencies were significantly longer than post-restraint controls (paired t-test, $p < .05$).

Writhing behavior induced by chemical irritants such as phenyl quinone or acetic acid is less well-defined in rat than in mice. Rats were observed for a period of 30 minutes following the intraperitoneal injection of irritant under blind conditions. A writhe consisted of body elongation followed by marked and prolonged contracture of the abdominal muscles. Pretreatment with TENS significantly reduced the number of writhes in treated rats (15.9 ± 9.8, n = 15) compared with untreated controls (31.6 ± 15.6, n= 15), $p < .05$, unpaired t-test.

Investigation of TENS-induced analgesia is being continued in other traditional pain models. Concurrent efforts are underway to define neurochemical correlates of TENS-induced analgesia.

141.10 DETERMINATION OF LEVELS OF MONOAMINE NEUROTRANSMITTERS AND THEIR METABOLITES IN REGIONS OF RAT CNS FOLLOWING ANALGESIA INDUCED BY TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION. K.M. Westphall* and J.A. Heltzel. Biosciences Research Lab., 3M, St. Paul, MN 55144.

Transcutaneous electrical nerve stimulation (TENS) has been shown to be efficacious for the relief of several types of clinical pain ranging from acute post-operative incisional pain to chronic low-back pain. In a companion abstract we report evidence for TENS-induced analgesia in several traditional pain models in the rat (Heltzel & Westphall, 1982). Preliminary studies in our laboratory have indicated changes in the level of some monoamines and their metabolites during TENS-induced analgesia in the rat. To date, only Wehne and Yucun (1981) have observed changes following low-frequency electroacupuncture applied transcranially in serotonin and norepinephrine in whole rat brain minus cerebellum.

Analgesia was evaluated in the heated bath/tail immersion paradigm by measuring tail flick withdrawal latencies before and immediately after the application of 30 minutes of TENS (100 Hz, 200 μS, ∿10 mAmps). Self-adhering electrodes were placed bilaterally at the base of the tail. Following the second test, animals were sacrificed by decapitation; 100-200 mg samples of brain and spinal cord were taken for quantitation by fluorometric methods. Unstimulated rats served as a source of control tissue.

Serotonin (5-HT) and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were extracted in acidified butanol and reacted with orthophthaldialdehyde to form fluorescent products, according to the method of Curzon and Green (1970). Norepinephrine (NE) and dopamine (DA) were quantitated fluorometrically using the method of Chang (1964) with slight modification following selective adsorption on alumina and elution into acetic acid. All results are expressed in terms of nanograms per gram wet weight (± SEM).

No change was demonstrated in the level of 5-HIAA, NE, or DA following TENS-induced analgesia in several rat brain or spinal cord regions: cerebral cortex, cerebellum, brainstem, upper cervical or lower lumbar cord. However, in three replicate studies, a significant 23% decrease in 5-HT in cerebellum was found in TENS-treated rats (100 ± 7, n=9) versus unstimulated controls (130 ± 10 n=6), $p < .05$ (Student's t-test). 5-HT content in all other areas examined remained the same. Two-way analysis of variance was run to assess day-to-day assay variability.

To the best of our knowledge, no one has examined regional changes in monoamine tissue levels following electrostimulation-induced analgesia. Others have implicated serotonergic mechanisms mediating peripheral electroacupuncture analgesia (Cheng and Pomerantz, 1981) and central stimulation-produced analgesia (Akil and Liebeskind, 1975). Furthermore, these same workers reported divergent results following catecholaminergic manipulations.

141.11 CORTICAL POWER SPECTRUM ANALYSIS OF PATHOPHYSIOLOGICAL PAIN. A.C.N. Chen & S.F. Dworkin. Pain Center; Dept. of Psychiatry & Beh. Sci.; Dept. of Oral Medicine, Univ. of Washington, Seattle, WA. 98195, U.S.A.

Brain activity as objective measures of human pain has recently became an intense focus of research. Prior studies (Chen et al., 1979; Carmon et al., 1981) show that exogenous noxious stimuli can evoke brain activity that is proportional to the painfulness experienced. Studies of brain activity during natural endogenous pain will provide further information on cortical mechanism of human pain. Although EEG has been recorded in clinical headache patients, few conclusive results have been consistently reported. Recently, advances in quantative EEG methods, such as the analysis of cortical power spectrum (CPS), have provided precise data suitable for statistical analysis of brain states.

This report describes the study of CPS in pathophysiologic pain, using acute dental pain as a model. Eleven 'walk-in' patients in an emergency dental clinic were recorded 10 min. prior to treatment for acute pain from tooth pulp pathology. One week later, 10 min. recording during non-pain states served as control. Subjective pain and anxiety scales were gathered before and after each recording. Each ten minutes of continuous CPS recording consisted of 10 spectrum/stage, 6 epochs/spectrum, and 10sec./epoch; each spectrum was stored, averaged, transformed, plotted by the Pain Microcomputer System (Dworkin et al., 1981).

Results of seven subjects, four of the elevens had confounding medications, showed significant cortical power reduction along all frequency bands (.5-50Hz) when pain states were compared to non-pain states. The magnitude of reduction also appeared to correspond to the subjective pain report. Rank order analysis of pain intensity suggests that cortical power changes in the alpha band may be inversely related to subjective painfulness, indicating that pain and α-desynchronization is closely associated. This study suggests that brain activity can be measured from clinical pain patients, and that the development of an objective pain measuring system seems feasible. Currently, we are studying other types of pathophysiological pain, post-operative pain, low-back pain, and the interactive effects on CPS of analgesic drugs and pain. The resulting additional analyses will be discussed.

141.12 MEASUREMENT OF PAIN IN RATS BY USE OF EVOKED POTENTIALS. C. G. Smith, S. Majidi-Ahi*, and N. Baxter*. Mount Holyoke College, Dept. of Biological Sciences, South Hadley, Mass. 01075

Epidural electrodes were surgically implanted over the frontal lobes of rats. Electroencephalographic recordings from the electrodes were digitized and averaged by a SYM microprocessor during programmed random repetitive stimuli. Thermal pain, electric shocks, and neutral sensory stimuli were used. A long latency waveform comparable to the "P-300" wave described in human subjects (Carmon, A., Mor, J. & Goldberg, J., Exp. Brain Res., 25:103, 1976) was observed. The evoked response could be transferred to a neutral sensory stimulus by conditioning. Morphine and enkephalin agonists selectively abolished the response. Rats treated neonatally with capsaican did not show the waveform.

It is proposed that this technique provides an objective measurement of the affective component of pain stimuli, and can be used as a measure of analgesia which avoids some of the problems inherent in behavioral tests such as the tail flick assay.

142.1 NASAL AND TEMPORAL HEMI-RETINA DIFFER IN THEIR GROWTH INTERACTIONS WITH GOLDFISH OPTIC TECTUM. J. Cronly-Dillon* and C.A. Stafford* (SPON: J. Kulikowski). Dept. of Ophthalmic Optics, U.M.I.S.T., Manchester, U.K.

Cronly-Dillon et al (1980) have shown that reciprocal growth regulating interactions take place between regenerating optic axons and tectal cells. Using changes in soluble tubulin and protein synthesis as indicators of cell growth interactions it was previously found that regenerating optic fibres stimulate biosynthetic changes in tectal cells and that the tectum exerts a retrograde growth promoting influence on the retina.

We have now compared the growth interactions induced by regenerating fibres from a nasal vs a temporal hemiretina and found that during the first stage of growth into the tectum, while growing axons are in the stratum opticum, the growth interactions induced between tectum and retina are significantly greater for a nasal hemiretina than for temporal hemiretina. Elsewhere Berry et al (1980) have shown that adhesive contacts between ingrowing axons and their target neurons induce changes in the growth and pattern of target neuron dendrites. Also Halfter et al (1981) have shown that chick retinal neurites from a nasal hemi-retina are more adhesive than those from a temporal hemi-retina. Thus the adhesive contacts established by the more adhesive nasal fibres appear to induce greater growth changes in tectal cells than their temporal counterparts. These and other results will be discussed in relation to retinotectal pattern formation.

Cronly-Dillon & Birks (1980) Adv. Physiol. Sci. Vol. 2. Regulating functions of the CNS subsystems (ed. J.Szentagothai, Hamori & Palkowitis).

Berry et al (1980) in 'Studies of normal and abnormal development of the nervous system (ed: Lierse et al, publ: S. Karger Berlin).

Halfter et al (1981) Nature 292, 67-70.

142.2 INDUCTION OF PEPTIDE-LIKE IMMUNOREACTIVITY (PLI) IN THE ANURAN OPTIC NERVE. R.O. Kuljis and H.J. Karten. Depts. of Psychiatry and of Neurobiology, S.U.N.Y. at Stony Brook, N.Y. 11794.

PLI has been shown in various classes of amacrine cells in vertebrate retinae. Ganglion cell layer elements, however, have been consistently negative (Brecha, Karten & Laverack,'79; Karten, Reiner and Brecha,'82). Interestingly, the anuran optic tectum displays a complex laminar pattern of PLI in an area which is in receipt of a massive retinofugal input (Kuljis & Karten,'81). Furthermore, retinal deafferentation is rapidly followed by marked reduction or dissapearance of most of the immunoreactive bands at this level (Kuljis & Karten,'82). Both latter findings suggest either a retinal contribution to PLI tectal laminar patterns or a transsynaptic effect.

Sixteen specimens of Rana pipiens were subjected to unilateral optic nerve ligation for one to 24 days. Substance P-, leucine-enkephalin-, cholecystokinin octapeptide- and bombesin-like immunoreactivity was analyzed in the retinae, optic nerves and optic tecti, on both sides, by means of the peroxidase-anti-peroxidase and fluorescence methods.

PLI is observed in the form of a dense array of fine longitudinal fibers, for all of the aforementioned substances, in the retinal stump of the optic nerve immediately adjacent to the ligature. The stain can be blocked by preabsorbing each of the antisera with the appropriate synthetic substance. No changes are observed in PLI patterns in the retina, nor is there any staining in the cerebral stump of the ligated nerve, nor in the contralateral optic nerve. The optic tectum contralateral to the ligation displays the changes we have described previously in retinal deafferentation experiments (Kuljis & Karten,'82).

These observations support the possibility that retinal ganglion cells contribute to PLI in the tectum, in spite of the fact that PLI cannot be normally demonstrated in their somata and processes. The clarification of this issue needs further experimental assessment. It is possible, in fact, that PLI observed in ligated optic nerves is a post-traumatic response, not reflecting a normal feature of retinal ganglion cells.

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142.3 ABILITY OF THE COBALT METHOD AND INABILITY OF THE RADIOAUTOGRAPHIC METHOD TO ADEQUATELY DETECT REGENERATING RETINTOTECTAL AXONS. A.D. Springer. Department of Anatomy, New York Medical College, Valhalla, N.Y. 10595.

In a previous study (Springer, JCN, 199:87, 1981) the distribution of regenerating goldfish optic axons was evaluated with [^{3}H]proline radioautography. Early in regeneration silver grains representing optic axons were concentrated in appropriate tectal laminae, but were also located in inappropriate tectal laminae. Later in regeneration, the silver grains were only observed in appropriate laminae. The early dispersion of silver grains in the tectum could represent transneuronal leakage of label from optic fibers in correct laminae to secondary tectal fibers. This study sought to determine whether label in inappropriate laminae represents label leakage or the presence of optic axons and whether regenerated axons in inappropriate tectal laminae are transient.

Cobaltous-lysine was applied to the regenerating optic nerve at various times following nerve crush (18-76 days post-crush with fish maintained at 20°C). Abnormally large numbers of cobalt-filled optic fibers were found coursing between the appropriate tectal laminae. Many aberrant fibers were located superficially in the stratum marginale and deep in the stratum periventriculare. The aberrant fibers were still apparent at the latest time point examined. Filled cells were not observed in the tectum, indicating that aberrant cobalt-filled fibers were optic axons and not transneuronally filled secondary processes.

The cobalt data suggest that: 1) radioautographic silver grains overlying the stratum marginale and stratum periventriculare probably represent optic axons and not leakage of labelled substances from regenerating axons, 2) transient axons as shown by radioautography are not transient when cobalt is used as a tracer; transient axons with radioautography may be related to regenerating axons carrying more label than regnerated axons, thereby making regenerated axons less detectable with radioautography, 3) the dispersion of axons throughout the tectal layers could reflect an abnormally large number of axons in the tectum during regeneration, 4) many regenerating fibers traverse tectal regions that they do not normally cross, 5) the final retinotectal projection may ultimately be carved from an abundance of regenerating fibers if the aberrant fibers eventually disappear and 6) the cobalt method is sufficiently sensitive to detect the presence of regenerating fibers at all stages of regeneration. (Supported by EY-03552)

142.4 RETINAL HISTOGENESIS AND THE DEVELOPMENT OF THE IPSILATERAL RETINO THALAMIC PROJECTION IN XENOPUS. S.G. Hoskins and P. Grobstein. Dept. Biol., Pharm. Phys. Sci., Univ. of Chicago, Chicago IL 60637.

A new retinofugal projection, that to the ipsilateral thalamus, develops in Xenopus during metamorphosis. We have studied the ontogeny of this projection and the histogenesis of the retina, to determine how the two processes are related.

We analyzed the development of the projection using anterograde transport of HRP applied to cut optic nerves. Labelling in ipsilateral terminal zones was first seen at tadpole stage 54. Between stages 54 and 66 (end of metamorphosis), reaction product density increased in ipsilateral thalamic terminal zones. Even in 1-6 month postmetamorphic frogs, however, the intensity of label was not comparable to that seen in adults. The immaturity of the projection at stage 66 was also evident in the distribution of label within the ipsilateral nucleus of Bellonci. In adults, label is fairly homogeneously distributed through this terminal zone. At stage 66, it was located in two subregions with an intervening label-sparse area. Labelling was similarly non-homogeneously distributed in 1, 2½, and 6 month postmetamorphic frogs.

To determine when ganglion cells giving rise to the ipsilateral projection are born, we injected tadpoles with ^{3}H-thymidine, let them metamorphose, and then injected HRP into the rostral thalamus to retrogradely label the ipsilaterally projecting ganglion cells. Since new cells are added primarily at the retinal periphery, the ^{3}H-thymidine injections at any given stage labelled the cells which at that time lay at the periphery. After metamorphosis, this ring of label separated earlier-born (central) from later-born (peripheral) cells. In tadpoles thymidine-injected before stage 54, nearly all HRP-filled cells were located peripheral to the thymidine ring. In cases injected at stage 54, HRP-filled cells were found in the vicinity of and peripheral to the ring. In cases injected at stages 56-58, significant percentages of HRP-filled cells lay central to the ring. We conclude that the vast majority of ipsilaterally-projecting cells are born at and after st. 54, the stage when an ipsilateral projection is first seen.

To determine whether postmetamorphic ganglion cell production could play a role in the postmetamorphic development of the ipsilateral terminal fields, we injected ^{3}H-thymidine into frogs at stage 66 and killed them several months later. Significant numbers of cells were evident peripheral to the labelled ring. Thus, cell addition continues postmetamorphically. Our previous retrograde HRP studies in adult frogs showed that ganglion cells at the extreme retinal periphery are among those which project ipsilaterally to the thalamus. We conclude that addition of ipsilaterally projecting ganglion cells continues postmetamorphically.

(Supported by NSF BNS 7914122)

142.5 RE-ESTABLISHMENT OF THE IPSILATERAL OCULO-TECTAL PROJECTION AFTER OPTIC NERVE CRUSH IN THE FROG: EVIDENCE FOR SYNAPTIC REMODELLING DURING REGENERATION. J.R. Adamson* and P. Grobstein. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

Following optic nerve crush, the crossed retino-tectal projection (CP) and the ipsilateral oculo-tectal projection (IP) in the frog are both re-established. During this process there is a phase when ganglion cell terminals in the CP are abnormally located. Whether these terminals form functional synapses is unclear. The IP is a map of the binocular visual field of one eye on the ipsilateral tectal lobe; it depends on the CP and a topographic intertectal relay. Whether the IP has a disorganized phase during regeneration is unknown. Since the visual field represented at a given locus in the IP reflects retinal input to a corresponding locus in the CP, functional synapses made by mislocated retinal terminals might be detected as abnormalities in the IP. To see if abnormalities exist, we have electrophysiologically mapped the CP and IP in Rana pipiens at various times after optic nerve crush.

A coarse topographic map was rapidly re-established in the CP, usually within about four weeks. The multiunit receptive fields (MRF's) were however greatly enlarged, with diameters up to three times normal. Single unit receptive fields and simultaneously recorded MRF's from the undisturbed eye were normal. We conclude that, at early stages of regeneration, terminals representing unusually wide areas of the retina are present at each tectal locus. MRF sizes declined to normal levels over about three months.

The re-establishment of the IP similarly involved the rapid appearance of a grossly topographic map with enlarged MRF's, and a later decline in MRF size. The sizes of IP MRF's were closely correlated with those of CP MRF's in the opposite tectal lobe at all times. Some IP MRF's seen at early times included part of the monocular field of the regenerating eye. The CP MRF's seen at the same time were expanded such that tectal loci normally receiving retinal afferents representing binocular visual field were also receiving input from those representing monocular field.

The re-establishment of the IP, like that of the CP, thus involves an initially disorganized map and a progressive recovery of order. The abnormalities in the IP closely reflected those of the CP, as would be expected if they result from effective synapse formation by mislocated retinal terminals. Consistent with this, enlarged IP MRF's could be abolished by small lesions at corresponding points in the opposite tectal lobe. Such lesions, as in normal animals, left the IP at surrounding tectal loci intact. It seems likely then that the observed changes in the IP during regeneration reflect a process of synaptic remodelling in the CP as the retinal area represented by ganglion cell terminals at particular tectal loci becomes progressively more restricted.

(Supported by PHS EY-01658 and RCDA EY-00057.)

142.6 ABERRANT CROSSED PARABIGEMINO-TECTAL PROJECTIONS IN RATS WITH NEONATAL RETINAL LESIONS. J.A. Stevenson and R.D. Lund. Dept. Anatomy, Medical Univ. of South Carolina, Charleston, S.C. 29425.

Previous work has shown that the axonal projections arising from the ipsilateral retina and contralateral parabigeminal nucleus (PBN) distribute to similar regions of the superior colliculus (SC) in the normal adult pigmented rat. Furthermore experimental animals which had one eye removed at birth, producing an enlarged ipsilateral retino-SC projection in the adult, have a similarly enlarged crossed PBN projection to that same SC. In bilaterally enucleated animals the PBN projection is not as greatly enlarged. These results indicate that there may be some form of interaction between retinal and PBN afferents to SC such that the retinal projection establishes conditions or boundaries which may govern the PBN terminal distribution (Stevenson and Lund, J. Comp. Neurol., 206:1982).

To examines the degree of precision of such interactions the present experiments have examined the influence of partially expanded ipsilateral retino-SC projections on crossed PBN projections to the same SC. Neonatal rats received partial lesions of their left retinae . At adulthood the projections from right eye and left PBN to right SC were assessed using ^{3}H-proline autoradiography and degeneration, respectively. Patches of aberrant ipsilateral retino-SC projection appeared in regions corresponding to gaps in the crossed retino-SC projection (Lund and Lund, Exp. Neurol., 40:37 1973). In those cases in which such abnormal patches of ipsilateral retinal projection appeared within the region of SC which normally receives crossed PBN afferents, there were spatially corresponding, localized areas of abnormally high density PBN projection. When no aberrant retinal projection was present, the PBN projection appeared normal.

This result indicates that the ipsilateral retinal projection does more than simply establish boundary conditions for crossed PBN termination in SC. The interactions between these two afferent projections may be very precisely localized and potentially useful in refining the topography of the PBN projection.

(Supported by Grants EY05381 and EY03414 from NIH).

142.7 MUTANTS AND CELL MARKERS IN XENOPUS LAEVIS: APPLICATIONS TO STUDIES OF EYE DEVELOPMENT. R. Tompkins*, D. Krotoski-Gwozdzowski* and R. K. Hunt*. (Spon: R. K. Murphey). Dept. of Biology, Tulane Univ., New Orleans, LA 70118 and Dept of Biophysics, Johns Hopkins Univ., Baltimore, MD 21218.

Surgical rearrangement of parts of eyebuds of amphibian embryos has long been used to define the developmental potentials of various parts of chimeric eyes. The lack of an adequate cell marker in these experiments led to controversial interpretations based on presumed fates of the chimeric parts. We have developed a fertile, stable, apparently normal strain of tetraploid(4n) Xenopus laevis which permits the identification of every cell in 2n/4n chimerae regardless of the state of differentiation of each cell. Diploid and tetraploid cells were distinquished by their cell size, nuclear size, nucleolar number, and DNA content.

We have used the 4n cell marker in heterotopic grafting strategies to demonstrate instances of regulative alterations in prospective retinotectal connections. 2n/4n chimerae have also been used to identify instances of both regulation and mosaicism in growth pattern in neural retina, pigmented retinal epithelium, and iris. Tetraploid cell size is sufficiently large during development to permit intracellular dye injections. This has led to studies of developing retinal neuron morphologies. Tetraploid embryos have been tolerized to one of our inbred diploid strains. This has permitted subsequent grafting of diploid neural tissues-- such as tectal fragments-- into the cell marked tetraploid host at any age without immunological complications. Finally, several mutants have been isolated which effect eye development. These include an undergrowing "tiny eye" mutant, an overgrowing "enlarged eye" mutant, and a neural cell lethal mutant which causes the autonomous death of neural cells just prior to feeding stage. These mutants, among others, not only provide interesting natural experiments for analysis but also permit the development of normal eye tissues -- either diploid or tetraploid -- to be challenged in subtle ways in chimeric eyes. Supported by NSF grants BNS 80-22451 to R. T. and BNS 77-26987 to R. K. H.

142.8 MOSAIC AND REGULATED PATTERNS IN RETINAL GROWTH AND RETINOTECTAL INNERVATION FOLLOWING HETEROTOPIC GRAFTING OF GENETICALLY-MARKED EYE-BUD FRAGMENTS IN XENOPUS. R.K. Hunt*, L.A. Faulkner*, B. Szaro*, K. Conway*, and R. Tompkins* (SPON: D. Fambrough). Biophysics Dept., Johns Hopkins Univ., Baltimore, MD. 21218 and Biology Dept. Tulane Univ., New Orleans, LA 70118.

The embryonic eye-bud in Xenopus frogs has a developmental program for patterned retinotectal connections, as revealed by 'mosaic' retinotectal patterns from embryonically grafted eyes and certain combinations of fused eye-bud fragments (rev. Curr. Tops. Develop. Biol. 15: 216, 1980). We recently confirmed, by exchanging genetically marked grafts between (pigmented) 4n embryos and (diploid) albino embryos, that the developmental program in grafted eye-bud tissue can be modified--leading to a 'regulated' normal retinotectal nerve pattern--following (i) surgical misalignment of whole eye-buds in body space at stage 26, or (ii) surgical recombination of a dorsal half-bud with an anterior half-bud at late optic cup stages (Biophys. J. 37, 59a, 1982). Here we report on wedge-grafts between two different angular positions on the stage 32+1 eye-bud (Anteroventral, AV vs. Dorsal, D), exchanged between (pigmented)4n donor embryos and (diploid)albino host embryos (in Rearing solution: 5% Steinberg-15% Holtfreter salines, MS-222 1: 5000 anesthesia, at 22^{o}). These wedge-grafts, which represent the full thickness of the eye-bud and 30+10^{o} in angular extent at surgery, heal within 72 hours. Orthotopic wedge-grafts (D-into-D,AV-into-AV) showed that each region had a characteristic groth pattern, revealed by the color marker in pigment retina (PR) and iris, and by the cell size marker in neural retina (NR). D-into-D grafts contributed little to iris; and their derivative sector in PR and NR showed limited addition of new cells from the ciliary margin at metamorphic stages. AV-into-AV wedge-grafts showed marked widening of their derivative sector, in iris during larval growth and in PR and NR added during metamorphic climax. All orthotopic-graft chimerae showed normal visuotectal projections, assayed electrophysiologically at termination after metamorphosis. AV-into-D heterotopic grafts gave two distinct classes of result. The AV-program was retained and expressed in one class (20%), so that (i) the angular territory of graft-derived tissue increased to 160^{o} in larval iris and post-metamorphic PR and NR, and (ii) the sector of visuotectal projection subtended by graft-derived NR mapped to a caudomedial triangle of tectum, duplicating the projection from the host's own AV-retinal sector. The second class (80%) showed 'regulated' normal visuotectal projection and a D-like growth pattern appropriate to the graft's new site of implantation: no widening of graft territory in larval iris, and limited addition of new NR and PR at metamorphic stages. We infer that pattern regulation affects both the mapping properties of the graft's derivative neurons and local programs for growth. Supported by NSF (BNS8022451; PCM7726987).

142.9 SINGLE CELL TECHNIQUES FOR THE DEVELOPING XENOPUS RETINOTECTAL SYSTEM. D.S. Sakaguchi*, R. Tompkins*, R.K. Hunt* and R.K. Murphey (SPON: H. Molinari). Dept. of Biol. Sci., SUNYA, Albany, N.Y., Dept. of Biol.,Tulane Univ., New Orleans, La.,and Jenkins Biophy. Lab, Johns Hopkins Univ., Baltimore, MD.

We have developed an in vitro preparation and applied intracellular dye injection techniques to the study of the developing Xenopus visual system. The animal we use is a strain of tetraploid Xenopus laevis developed by one of us (R.T.). The retinal ganglion cells in tadpole stages of these animals are larger in diameter than their diploid counterparts (15 vs 8u respectively). The experimental preparation consisted of isolating the retina and after removal of the lens and pigment retinal epithelium, viewing the ganglion cells from the vitreal surface. The preparation was transilluminated and viewed with Nomarski optics. Ganglion cells were impaled with microelectrodes filled with Lucifer Yellow or hexamminecobalt chloride and examined in animals between stages 26-50 (Nieuwkoop and Faber, 1956). A broad continuum of morphologies was observed. The earliest cellular profiles were of neuroepithelial form (stages 25-28), irregular in shape and bearing few if any processes. Cells examined at later stages (after stage 28) ranged from unipolar to multipolar and exhibited increasing dendritic complexity with age. The earliest we saw "mature" dendritic forms was at stages 39/40.

As a complement to the single cell techniques we filled ganglion cell axons by immersing an isolated brain in a pool of hexamminecobalt chloride with optic nerves and eyes still attached, but outside the pool of staining solution. After a diffusion period (10 hours) the eyes were processed as wholemounts and the cobalt intensified according to the procedure of Bacon and Altman (1977). The ganglion cells could be divided into two general classes based on soma size, large cells constituting less than 10% of the total population and a small class constituting the remainder. Within these two classes we observed a variety of dendritic patterns. Data regarding axon fasciculation confirmed the gross radial order of optic axons, but just as the single cell technique has demonstrated it is variable. A small percentage of ganglion cell axons do not merge with adjacent fascicles but instead merge with distant fascicles, thereby taking an indirect route to the optic disc. We are currently extending the intracellular technique to an isolated eye, optic nerve and brain preparation in an attempt to fill single ganglion cells from the eye to the brain. By examining both ends of the ganglion cells we hope to provide high resolution techniques for the continued study of the retinotectal system.Supported by NSF Grants to R.T., R.K.H. and R.K.M.

143.1 EFFECT OF AF64A ON NEUROBLASTOMA X GLIOMA HYBRID CELL LINE NG-108-15: A NEUROTOXIN SELECTIVE FOR CHOLINERGIC CELLS. K. Sandberg*, R.L. Schnaar, I. Hanin, and J.T. Coyle. (SPON: L. Tune,) Div. of Child Psychiatry, Depts. of Psychiatry, Pharmacology and Neuroscience, Johns Hopkins Univ. School of Med., Baltimore, MD and Dept. of Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA.

Intraventricular injection of the ethylcholine mustard aziridium ion (AF64A) induces a persistent and significant reduction in acetylcholine (ACH) levels and in the choline high affinity uptake process in mouse brain (Mantioni et al., Science 213:579, 1981). We have previously demonstrated a selective reduction in markers for cholinergic neurons in the striatum without reduction in presynaptic markers for other neuronal populations after in situ injection (Coyle, et al., Trans Am. Soc. Neurochem 13:271, 1982). In the present study, AF64A's effect on the neuroblastoma x glioma hybrid cell line, NG-108-15, was examined in an attempt to elucidate the neurotoxic mechanism of this drug. NG-108-15 possesses a high affinity choline uptake system (7.8 pm/mg prot-/min) and choline acetyltransferase activity (CAT) which trebles upon differentiation (from 133 to 377 pmole/mg prot/min). These cells release ACH upon depolarization and form neuromuscular synapses in culture. (Hamprecht, Int.Rev.Cytol. 49:99, 1977).

Cytotoxic effects of the drug were monitored by the release of lactic dehydrogenase (LDH) into the incubation medium. AF64A caused cell death of NG-108-15 within 48 hours at concentrations from 50-200 μM. Differentiated NG-108-15 showed a three-fold increased susceptibility to the cytotoxic action of AF64A as compared to undifferentiated NG-108-15 and to a non-cholingeric neuroblastoma cell line. A kinetic study indicated a 70% decrease in specific activity of CAT after 2.5 hours of exposure to 100 μM AF64A; at this time point, LDH activity in the cells or in the medium was not significantly different from control. Cytotoxic effects with increased LDH activity in the medium appeared over the subsequent twenty-four hours. Hemicholin- ium-3, the potent antagonist of the choline high affinity uptake process, suppressed the toxic effects of AF64A in a dose-related manner. The results of these studies indicate that AF64A exhibits selective cytotoxic effects against this cholinergic cell line that is dependent upon the activity of the choline transport process; furthermore, inhibition of CAT activity is an early event that precedes the cytotoxic action of the drug. This compound appears to be a cholinergic-specific neurotoxin and offers promise as a tool for selectively lesioning cholinergic systems. (This research is supported by USPHS Grant NS-18414 and KS is supported by NIH Training Grant GM 07445).

143.2 IOTROCHOTIN, A NOVEL FACTOR FROM A CARIBBEAN SPONGE, INCREASES RELEASE OF NEUROTRANSMITTERS. J.V. Martin and W.O. McClure. Dept. Biol. Sci., Univ. Southern Calif., Los Angeles, CA 90007.

Several species of marine organisms collected during the 1978 Alpha Helix Caribbean Expedition have been identified as sources of substances which affect the release of acetylcholine and choline from a synaptosomal preparation (Martin, J.V. and W.O. McClure, Trans. Am. Soc. Neurochem., 11:85, 1980). One sponge in particular, Iotrochota birotulata, has been selected for more intensive study. Extracts of this organism contain a molecule which can, at high doses, release all radioactivity from synaptosomes preloaded with tritiated choline. The active agent, or "iotrochotin" (IOT), has a molecular weight of approximately 12,000 as determined by Sephadex G50 chromatography, used in these studies as a crude purification step. IOT is completely inactivated by heating at 90°C for 5 min, but is resistant to treatment with trypsin. Release of radioactivity shows a sigmoid dependence on the dose of IOT, but the data do not generate a linear Hill plot. The time course of action is extremely rapid, and is complete by the earliest time point measured, at 1 min.

The mechanism of action of IOT does not appear to be a simple direct effect on trans-membrane fluxes of either Ca^{2+} or Na^{+}. Removal of Ca^{2+} from and addition of 1 mM EGTA to the synaptosomal incubation medium reduces the effect of IOT by 28%, although this treatment dramatically inhibits release due to electrical stimulation. Removal of Na^{+} from the incubation medium reduces release due to 10^{-4} M veratridine by 57%, but inhibits the effect of IOT by only 14%. In addition, tetrodotoxin inhibits veratridine induced release of radioactivity by 95%, while inhibiting the effect of IOT by 12%. Finally, if ion fluxes were major components of the action of IOT, one might expect that depolarization of the synaptosomal membrane would result. The average change in membrane potential of synaptosomes was measured using the fluorescent dye DiO-C_5-(3), and there was no effect of a dose of IOT which would have caused a major release of radioactivity.

Not only cholinergic synaptosomes can be influenced by IOT. Synaptosomes preloaded with tritiated norepinephrine or gamma-aminobutyrate also released radioactivity as a result of treatment with IOT. It is felt that IOT is a toxin which interacts with a presynaptic mechanism other than the regulation of metal ion fluxes but common to systems of neurotransmitter release. When purified, IOT may be a useful tool in studying mechanisms of release. (Supported by NSF Grants BNS 76-80657 and BNS 77-06782, the Dreyfus Foundation, and Nelson Research and Development Co., Irvine, CA.)

143.3 MONOCLONAL ANTIBODIES TO RAT BRAIN CHOLINE ACETYLTRANSFERASE William L. Strauss* and Marshall Nirenberg. (SPON: M.D. Schneider) Lab. Biochemical Genetics, NHLBI, NIH, Bethesda, MD.

Four monoclonal antibodies have been prepared to rat acetyl CoA: choline O-acetyltransferase (EC 2.3.1.6, CAT). The enzyme was purified approximately 100,000-fold from whole rat brain by precipitation with acetic acid at pH 4.5, fractionation with 40-60% $(NH_4)_2SO_4$, CM-Sephadex chromatography (Ryan and McClure (1979) Biochemistry, 18: 5357) and affinity chromatography on coenzyme A-hexane-agarose. The enzyme preparation was applied to the affinity column in the presence of 10 mM acetylcholine to increase the affinity of CAT for coenzyme A and eluted with 10 mM acetyl coenzyme A. BALB/c mice were immunized with the purified enzyme (greater than 60% CAT estimated after SDS-PAGE). Fusion of spleen cells with P3X63· Ag8 myeloma cells resulted in 4 CAT-specific colonies (anti-CAT 1-4) out of 93 hybridomas. Hybridomas directed towards CAT were identified by their ability to precipitate the enzyme in the presence of protein A at pH 8.5. Incubation of anti-CAT 1-4 with CAT followed by precipitation with rabbit anti-mouse IgG and protein A on formalin-fixed S. aureus reduced enzyme activity in the supernates 38, 26, 30 and 34%, respectively. This amount of precipitation is consistent with the value calculated, using the law of mass action, from estimates of the reactant concentrations (CAT, 3.5 x 10^{-10} M; hybridoma antibody, 3.3 x 10^{-8} M) and of the equilibrium dissociation constant (10^{-7} M). A mixture of the four antibodies (each at 25% of the usual concentration) precipitated 40% of the enzyme activity. The ability of conditioned medium to precipitate CAT was reduced greater than 50% by prior treatment with protein A to remove antibodies. Anti-CAT 1, 2 and 3 inhibited the enzyme to the same extent that each precipitated activity. Anti-CAT 4 was less effective in inhibiting CAT (21%) than in precipitating the enzyme (34%). Hybridoma antibody-dependent enzyme inhibition was reversed by dissociation of immune complexes via dilution. CAT activity was partially recovered from [CAT · anti-CAT · rabbit anti-mouse IgG· Protein A] immune precipitates by suspending the pellets in fresh buffer for 3 hr. The specificity of the hybridoma antibodies was confirmed further by two dimensional gel electrophoresis of immune precipitates. Each of the four antibodies selectively precipitated a protein with a molecular weight of 67,000, corresponding to the value for affinity-purified CAT. Anti-CAT 1, 3, and 4 cross reacted with 14-day embryonic chick brain CAT, precipitating 46, 15 and 19% of the enzyme, respectively, suggesting that portions of the enzyme were conserved during evolution. These antibodies should be useful for the identification of mRNA coding for CAT and for the generation of cDNA probes for the choline acetyltransferase gene.

143.4 IMMUNOHISTOCHEMISTRY OF CHOLINE ACETYLTRANSFERASE IN THE RAT BRAIN M. V. Sofroniew[1], F. Eckstein[2]*, H. Thoenen[2] and A. C. Cuello[1]*. [1]Departments of Human Anatomy and Pharmacology, University of Oxford, South Parks Road, Oxford OX1 3QX, U.K.; [2]Max Planck Institute for Psychiatry, Dept. of Neurochemistry, Martinsried, FRG.

The enzyme choline acetyltransferase (ChAT) is responsible for the synthesis of acetylcholine and currently represents the most useful marker for cholinergic neurons. In this study, cholinergic neurons were examined in the rat brain by immunohistochemical detection of ChAT. ChAT was purified from pig brain and antisera and monoclonal antibodies were generated against ChAT of over 95% purity as previously described (Neurosci., 6:993, 1981; EMBO J., 1:in press, 1982). The monoclonal antibody generated was useful for immunohistochemical detection of ChAT only in the pig CNS. However, several antisera showed immunohistochemical reactivity with ChAT of other species. These antisera gave staining identical to the monoclonal antibody when compared in the pig CNS, and various other tests verified their monospecific nature (EMBO J., 1:in press, 1982). Detailed immunohistochemical studies were conducted using Wistar rats 220-280 g fixed by perfusion with 4% paraformaldehyde/0.01% glutaraldehyde. Vibratome sections 25 or 50 μm thick were prepared and immunohistochemically stained free-floating as previously described (Neurosci., 6:619, 1981). As expected, motor neurons in the ventral horn of the spinal cord and nuclei of the cranial nerves were positively stained. Preganglionic sympathetic neurons in the intermediolateral nucleus were also stained. In the forebrain, a large number of ChAT-positive neurons was found forming a continuous group in the nucleus of the diagonal band of Broca and medial septum. Many ChAT neurons were also present in and around the area of the medial forebrain bundle and nucleus basalis (Meynert) and intermingled in the white matter at the ventral border of the internal capsule and striatum. In the neostriatum, many single ChAT neurons were evenly scattered throughout the caudatoputamen and groups of ChAT neurons were found intermingled in the white matter along the medial border of the globus pallidus. In the midbrain, ChAT neurons were present in the red nucleus and Edinger-Westphal nucleus. ChAT neurons in most areas were large (20-30 μm). Although ChAT neurons and their proximal processes were well visualized in this study, terminal fields and fiber tracts were not well stained. In some cases, such as the interpeduncular nucleus, a diffuse dark staining was noted throughout the neuropil of a circumscribed area, but could not clearly be defined to punctate fibers or terminals. Thus, although work remains to be done on improving the visualization of cholinergic terminal fields, neural perikarya in certain areas (listed above) can unambiguously be identified as cholinergic. (Supported by NIH NRSA 1 F32 NS06956-01, the Wellcome Trust and the Deutsche Forschungsgemeinschaft.)

143.5 APPLICATION OF A SELECTIVE RETROGRADE LABELING TECHNIQUE TO THE IDENTIFICATION OF ACETYLCHOLINE SUBCORTICOSPINAL NEURONS. M.F. Paré, B.E. Jones and A. Beaudet. Lab. of Neuroanatomy, Dept. of Neurology and Neurosurgery, Montreal Neurological Inst., McGill University, Montreal, Quebec H3A 2B4.

It has been proposed that neurons may be distinguished according to their neurotransmitters by selective terminal uptake and retrograde axonal transport of their radiolabeled transmitter. Recently Bagnoli et al. (J. Neurosci. 81: 691-695, 1981) demonstrated selective retrograde radioautographic labeling of acetylcholine neurons in the septum following injection of ^{3}H-choline into the hippocampus. In the present study the same technique has been applied to the identification of brain stem acetylcholine neurons which project to the spinal cord.

^{3}H-choline (10-100µCi of 80 Ci/mmol in 0.1-0.5µl distilled water) was injected through a glass micropipette into the first right cervical segment of the spinal cord in rats anesthetized with Nembutal. The animals were sacrificed 20 hours later by perfusion of an ice cold solution of 10mM Hepes buffer containing 110 mM Mg^{++} followed by a 4% paraformaldehyde solution in the same buffer. The brains were rapidly removed and frozen with dry CO_2 gas. Twenty µm-thick sections were cut in a Cryostat, touch mounted on gelatinized slides, dried with a blow dryer, and processed for radioautography of diffusable substances by apposition of emulsion pre-coated coverslips.

Two patterns of radioautographic labeling were evident in the brain stem following spinal injections of ^{3}H-choline. First, anterograde axonal and terminal labeling which is known not to be specific for cholinergic neurons was present in spinoreticular, spinocerebellar, and spinothalamic pathways. Second, retrograde axonal and perikaryal labeling was evident in certain subcorticospinal systems. In the medulla, nerve cell bodies within the nuc. reticularis gigantocellularis, nuc. raphe magnus and the medial, inferior and lateral vestibular nuclei were labeled on the ipsilateral and to a lesser extent contralateral side. In the pons, a few cell bodies within the nucleus reticularis pontis caudalis were detected. In the midbrain, cells of the contralateral red nucleus were heavily labeled. Many regions known to project to the cervical spinal cord, such as the nuc. reticularis parvicellularis and the nuc. reticularis pontis oralis contained no labeled cells. Most significantly the noradrenaline locus coeruleus neurons which project to this region were not labeled. In view of this demonstration of selective retrograde labeling with ^{3}H-choline, it is proposed that certain rubrospinal, vestibulospinal and medial medullary reticulospinal cells may be cholinergic. (Supported by a studentship and grants MA 6464 and MA 7366 from the Medical Research Council of Canada).

143.6 ACETYLCHOLINESTERASE-CONTAINING NUCLEUS BASALIS NEURONS OF THE LATERAL HYPOTHALAMUS PROJECT TO THE SPINAL CORD. J. H. Haring* and J. N. Davis (SPON: E. Busse). Neurology Research Laboratory, Veterans Administration Medical Center, Durham, NC 27705 and Departments of Medicine (Neurology) and Pharmacology, Duke University, Durham, NC 27710.

Acetylcholinesterase-containing neurons of the basal forebrain are arranged in a discontinuous column extending from the medial septum to the lateral hypothalamus, and may represent a highly collateralized cholinergic analog of the catecholaminergic locus coeruleus projection. Although the locus coeruleus is known to have spinal projections, no comparable projection has been identified for cholinergic neurons of the basal forebrain. Therefore retrograde transport of horseradish peroxidase (HRP) or the fluorescent dye bisbenzimide (Bzb) and acetylcholinesterase (AChE) histochemistry were used to test the hypothesis that basal forebrain cholinergic neurons project to the spinal cord.

Large, nonspecific injections of either 10% Bzb or 50% HRP were made at middle thoracic levels of the spinal cord. In several rats, HRP crystals were applied to lesions of the thoracic cord. Following 48 hour survivals, numerous fluorescent neuronal nuclei and HRP-filled perikarya were seen in regions known to have a conspicuous projection to the spinal cord (e.g. motor cortex and red nucleus). In contrast to the sparse HRP labeling observed in the paraventricular nucleus, numerous Bzb labeled nuclei were observed in neurons of not only the paraventricular nucleus but the lateral hypothalamus as well, thus demonstrating a differential sensitivity of these projections for Bzb and HRP. Sections having Bzb labeling were photographed, stained for the presence of AChE and the identical regions rephotographed in order to compare retrograde labeling with AChE activity. Although AChE staining often did not correlate with Bzb fluorescence, a small number of fluorescent neuronal nuclei appeared to correspond to AChE-containing perikarya in the lateral hypothalamus. The observation of these retrogradely labeled, AChE-positive neurons suggests a small descending cholinergic pathway originating in neurons of the basal forebrain.

Further support for the hypothesized cholinergic basal forebrain-spinal cord projection is found in the appearance of sympathetic fibers in the spinal cord distal to a complete transection. This phenomenon has also been reported to follow lesions of other cholinergic forebrain nuclei (medial septal nucleus, ventral pallidum), and may represent a common response which follows the destruction of any constituent of the basal forebrain cholinergic column.

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143.7 ACETYLCHOLINE AND γ-AMINOBUTYRIC ACID SYNTHESIS BY DISSOCIATED CEREBRAL CORTICAL CELLS IN VITRO. W.E. Thomas. Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Primary cultures were prepared from dissociated embryonic rat cerebral cortex. The cortical cells attained a mature appearance after approximately 3 weeks in vitro and cultures were routinely maintained for 6-8 weeks. Mature cultures contained both neurons and nonneuronal cells. The presence of neurons in such cultures was determined by two methods, indirect immunofluorescence of tetanus toxin binding and staining with the excitable-cell-specific dye merocyanine 540.

The synthesis of acetylcholine (ACh) and γ-aminobutyric acid (GABA) was determined by incubating cultures with ^{3}H-choline or ^{3}H-glutamate for 10 min. The cultures were then extracted and the extract analyzed by high voltage electrophoresis. The radioactivity comigrating with standard ACh and GABA was quantified. In the presence of labelled choline, the cultures accumulated choline and synthesized ACh. Choline uptake was significantly reduced by 50 µM hemicholinium-3. The synthesis of ACh by intact cultures was inhibited 50-80% by naphthylvinylpyridine (250 µM); however, in lysed cultures, greater than 90% of ACh synthesizing activity was inhibited by this compound. The rate of ACh synthesis increased linearly with increasing concentrations of choline and began to saturate near 50 µM choline. Synthesis of GABA from labelled glutamate was also detected. This synthesis was linearly related to glutamate concentration and began to plateau in the range of 25 µM glutamate. The synthesis of ACh and GABA from 5 µM choline and 50 µM glutamate, respectively, was linear for at least 30 min. Autoradiographic studies demonstrated specific accumulation of labelled GABA by a subpopulation of neurons. GABA uptake was almost completely eliminated by diaminobutyric acid (1 mM) but unaffected by β-alanine (2 mM).

The synthesis and accumulation of both compounds from their respective precursors showed a similar dependence on culture age; the rate of synthesis increased constantly during the first 3 weeks the cortical cells were in culture, thereafter, it declined. The addition of muscle or various other nonneuronal cells caused only a slight enhancement in the ability of cortical cells to synthesis ACh and GABA. The synthesis of ACh and GABA is interpreted to be supportive of the use of these compounds as neurotransmitters by cortical neurons in vitro. Various lines of evidence have supported the presence of GABAergic neurons in the cerebral cortex in vivo; however, the existence of cholinergic neurons is less certain. The presence of cholinergic function in culture could reflect either the intrinsic presence of cholinergic cortical neurons or the result of developmental influences on a subpopulation of neurons.

143.8 SIMULTANEOUS DETERMINATION OF ACETYLCHOLINE AND CHOLINE IN NEURONAL TISSUE BY HPLC WITH ELECTROCHEMICAL DETECTION. P. E. Potter*, J. L. Meek and N. H. Neff. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

We have developed a rapid, simple, sensitive, and specific method for the determination of acetylcholine (ACh) and choline (Ch) using HPLC with electrochemical detection. The method is based on the separation of ACh and Ch by reverse phase HPLC and mixing the effluent with acetylcholinesterase and choline oxidase, which converts choline to betaine and 2 H_2O_2. Production of H_2O_2 is continuously monitored electrochemically.

ACh and Ch are separated on a 15 cm Bio-Rad Bio-Sil ODS-5 column with a mobile phase of 0.01M sodium acetate buffered to pH 5 with 0.02M citric acid and containing 0.0025M tetramethylammonium chloride and 4 mg/L sodium octyl sulfate, at a flow rate of 0.8 ml/min. A solution of 0.2M sodium phosphate, pH 8.5, containing acetylcholinesterase (Sigma, 2 U/ml) and choline oxidase (Sigma, 1 U/ml), is added to the effluent from the column at a flow rate of 0.5 ml/min. Ch and ACh are converted to H_2O_2 in a postcolumn reaction coil (30 m of 30 gauge Teflon tube to give a delay of 2.5 min). The H_2O_2 produced is detected by oxidation at a potential of +0.5V across a platinum electrode in a Bio Analytical Systems LC4-A electrochemical detector.

Under these conditions the retention times of Ch and ACh are 4.5 min and 13.5 min respectively. 3-Dimethylamino-1-propanol (retention time 5.5 min) may be used as an internal standard. If determination of only ACh is desired, sodium octyl sulfate can be omitted from the mobile phase and the concentration of tetramethylammonium chloride increased to 0.005M. This reduces the retention time of ACh to 4 min.

The sensitivity of detection with this method is 1 pmol for Ch and 2 pmol for ACh. When pure acetylcholinesterase is used, choline esters such as butyrylcholine, α-methylacetylcholine, valerylcholine and phosphocholine are practically undetectable.

ACh and Ch levels in hippocampal extracts from rats killed by microwave irradiation were .19±.02 and .34±.04 nmol/mg protein (±S.E.M.) respectively. These correspond to levels obtained by gas chromatography-mass spectrometry (Costa et al., J. Clin. Pharmacol. 21:2565, 1981).

HPLC-EC offers many advantages over conventional methods for determination of ACh. Both ACh and Ch can be measured in the same sample, yet it uses equipment commonly found in many laboratories. Because no prior derivatization or separation of ACh and Ch is required, sample preparation is rapid. The method is simple and reproducible and offers excellent sensitivity and specificity.

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143.9 THE CHOLINE CYCLE: EVIDENCE FOR BIOSYNTHESIS OF CHOLINE FROM SERINE AND METHIONINE IN RAT BRAIN. J.K. Blusztajn* and R.J. Wurtman
M.I.T., Cambridge, MA 02139

We have previously shown that bovine and rat brain synaptosomes synthesize phosphatidylcholine (PC), by sequential methylation of phosphatidylethanolamine (PE), catalyzed by an enzyme, phosphatidylethanolamine N-methyltransferase (PeMT), which utilizes S-adenosylmethionine (SAM) as a methyl donor. PC synthesized by this pathway (apparent Vmax = 17 pmol/mg protein/10min.) has a much shorter half-life than other, previously-described synaptosomal pools of PC, such that after 30 min. of incubation 30% of the newly-formed PC was hydrolyzed to free choline (Ch). The specific activity of the newly-formed Ch was 88 ppm, whereas that of the newly-formed PC was only 2 ppm, so that the relative enrichment of the synaptosomal Ch pool was 44-fold higher than that of PC. The rate of PE methylation was markedly stimulated by monoamines (Leprohon et al. in this volume).

When synaptosomes were incubated with 20 μM [^{14}C-U]-serine (S), incorporation of this amino acid into phospholipids proceeded linearly for 10-15 min. After 10 min. of incubation the radioactivity in the ethanolamine moiety of PE was 1.25 fold higher than that of S moiety of the phosphatidylserine (PS) (1.4 pmol/mg protein). (The incorporation of S into phospholipids was stimulated by Ca++ and Ni++ and was inhibited by Zn++; monoamines had no effect on this process; it could be increased ten-fold by increasing the serine concentration.) Thus the 14C-S-derived PS is rapidly decarboxylated to PE. This PE may serve as a substrate for the PeMT.

We conclude that rat brain synaptosomes synthesize Ch de novo utilizing S and methionine (M) as its ultimate precursors. S serves as a donor of the ethanolamine portion of the Ch molecule and M, after conversion to SAM, donates the three methyl groups. Some of this newly-formed Ch may be available for neuronal acetylcholine synthesis. It is possible that the incorporation of S into PS and the liberation of Ch from PC is catalyzed by base exchange enzyme, such that the phosphatidic acid moiety of the PS and PC is recycled.

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143.10 THE IMMUNOHISTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE-LIKE IMMUNOREACTIVITY AT THE LIGHT AND ELECTRON MICROSCOPIC LEVELS WITHIN THE CENTRAL NERVOUS SYSTEM OF THE GUINEA PIG. B. Maley, R. Elde, B. Wainer and A. Levey Univ. of Minnesota Sch. of Med., Minneapolis, Mn. 55455 and Univ. of Chicago, Chicago, Il. 60637

Monoclonal antibodies raised in response to the bovine form of choline acetyltransferase (ChAT) have been previously demonstrated to be specific for the enzyme and to exhibit cross-reactivity to a variety of mammalian forms using biochemical methods (Levey et al., Brain Res., 234:469,1982). It was the purpose of the present investigation to study its use as a immunohistochemical marker in one species, the guinea pig. Using a modification of the peroxidase, anti-peroxidase (PAP) method we have identified ChAT-like immunoreactivity throughout the central nervous system of the guinea pig.

Subsequent to a perfusion of 4% paraformaldehyde-0.1% glutaraldehyde in 0.1M phosphate buffer, 100 μm coronal sections of several guinea pig brains were stained using anti-ChAT, followed by the PAP procedure, reacted with 3,3'-diaminobenzidine and hydrogen peroxide. The sections were then either dehydrated and mounted on gelatin coated slides or embedded in plastic for ultrastructural studies. Control sections incubated either with rat IgG or without the primary antibody were run concurrently with the remainder of the tissue and typically lacked immunostaining.

Examination of the sections revealed an extensive network of immunostained neurons throughout the rostrocaudal extent of the guinea pig brain. Nuclear regions containing ChAT immunostained neurons included the nucleus of the diagonal band of Broca, caudate nucleus, putamen and all the cranial nerve motor nuclei. In addition, immunostained fibers were visible as fascicles of cranial nerve motor nuclei and as diffuse fibers within the cortex. At the ultrastructural level ChAT immunoreactivity could be found within the neuronal cell body and large primary dendrites.

Results of the present investigation indicate a network of ChAT immunostained neurons using a monoclonal antibody in the guinea pig similar in distribution to those previously described in the cat and the rat. In addition, we have demonstrated that the monoclonal antibody to ChAT does recognize the enzyme from other species.

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143.11 TOPOGRAPHIC ANALYSIS OF THE INNERVATION OF THE RAT NEOCORTEX AND HIPPOCAMPUS BY THE BASAL FOREBRAIN CHOLINERGIC SYSTEM. M. McKinney*, J. Hedreen* and J.T. Coyle. Div. of Child Psychiatry and Depts. of Neuroscience, Psychiatry, Pathology and Pharmacology, Johns Hopkins School of Medicine, Baltimore, MD 21205

The innervation of rat cerebral cortex (Cx) and hippocampus (Hip) by a contiguous system of large neurons in the basal forebrain located in the globus pallidus (GP), lateral preoptic area (LPA), diagonal band (DB) and medial septum (MS) was topographically mapped by retrograde tracer methods with horseradish peroxidase (HRP) and with fluorescent dyes, fast blue (FB) and nuclear yellow (NY). Acetylcholinesterase (AChE) histochemical staining was performed 5 hrs after pretreatment with an AChE inhibitor to reveal AChE reactive cell bodies in the basal forebrain complex. In addition, the effects of excitotoxin lesions to the rostral and caudal aspects of the basal complex on the specific activity of choline acetyltransferase (CAT) was determined in subareas of the neocortex and in the Hip.

Excitotoxin lesions affecting the MS and DB caused a 64%* reduction in CAT activity in the Hip, 32%* in the occipital Cx, 13%* in the lateral Cx and 6% in frontal cortex; in contrast, lesion of the ventral medial GP caused a 59%* decrease in CAT in the frontal Cx, 50%* in the lateral Cx, 20%* in the occipital Cx and 7% in the Hip. Discrete injections of retrograde tracers in the various areas of the Cx and Hip revealed a gradient of distribution of labeled neuronal cell bodies in the basal forebrain complex consistent with the lesion studies. While labeled cells were noted virtually throughout the basal forebrain complex after discrete injections in the frontal Cx on one hand or the Hip on the other, the former caused the greatest percentage of labeled cells located in the GP whereas the latter was associated with the greatest percent of labeled cells in the MS/DB. Collatoralization of these projections was further demonstrated in studies of separate and non-overlapping injections of FB and NY in the frontal Cx and in the parietal Cx. Consistent with results from composite maps of single injection cases, the distribution of FB and NY labeled cells in the GP overlapped after the dual injections. Cell counting techniques indicated that nearly 20% of the retrograde labeled cells in the GP were unequivocally dually labeled with NY and FB. Thus, we conclude from these studies that the neurons within the basal forebrain cholinergic complex exhibit a topographically organized pattern of innervation of the Cx and Hip but that a significant degree of collatoralization of these projections occurs. (This research was supported by USPHS Grants NS-18414, MH-00125 and grants from AFAR and the McKnight Foundation).

*$p < 0.05$.

143.12 KINETIC STUDIES OF CHOLINE ACETYLTRANSFERASE AND MUSCARINIC RECEPTOR BINDING IN AGED C57BL/6J MICE. Steven B. Waller* and Edythe D. London (Spon: J.E. Moreton) Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore City Hospitals, Baltimore, Maryland 21224.

We have demonstrated previously that choline acetyltransferase (ChAT) activity is higher in the cerebral cortices, striata and hippocampi of aged C57BL/6J mice than in the same brain regions of young mice (S.B. Waller, D.K. Ingram, M.A. Reynolds and E.D. London, Abstr. Soc. Neurosci. 7:186, 1981). The current investigation was initiated to determine if these increases in regional ChAT activities reflect changes in the Km's for substrates or in Vmax, and whether they are associated with changes in muscarinic receptor binding.

Specific activities of ChAT were determined radiometrically in brain regions of 6-, 12- 18- and 24 month old male C57BL/6J mice. The effects of substrate concentrations on ChAT activity were studied by varying the concentrations of acetyl-CoA and choline chloride in enzyme incubations. Km and Vmax were calculated by the Eadie-Hofstee method. The densities of muscarinic binding sites (Bmax) and the ratio of high to lower affinity binding sites were measured with [3-H]quinuclidinyl benzilate as the ligand in displacement assays with carbamylcholine, as previously described (M. McKinney and J.T. Coyle, J. Neurosci. 2:97,1982).

Kinetic studies of ChAT activity revealed that age differences were due to elevated Vmax values in older mice. These age-related increases in Vmax for ChAT in the cerebral cortices and striata were associated with significant reductions in muscarinic receptor densities. For example, in the cerebral cortices, the Vmax for ChAT was significantly higher in 18- and 24 month old mice than in younger mice, while the total muscarinic receptor density was significantly reduced in 18- and 24 month old mice compared with values in younger animals. However, in the hippocampus, both the Vmax for ChAT and the Bmax for muscarinic receptor binding were significantly higher at 24 months than in younger animals. There were no age differences in the ratios of high to lower affinity muscarinic binding sites.

The results indicate that with aging in the C57BL/6J mouse, regional changes occur in the concentration of ChAT protein and density of muscarinic receptors. There is no evidence for changes in affinities for the enzyme substrates or receptor ligands. Further studies may help elucidate the relationships between age effects on these pre- and postsynaptic markers for cholinergic transmission.

144.1 EVIDENCE FOR TWO KINDS OF ACETYLCHOLINE RELEASE BY AN IDENTIFIED RETINAL NEURON. Richard H. Masland, Massachusetts General Hospital, Harvard Medical School, Boston MA 02114.

The cholinergic neurons of the rabbit retina make up a small subset of the amacrine cells. They are positioned with mirror symmetry lining the inner synaptic layer, about half of them in the conventional position of amacrine cells and half "displaced" to the ganglion cell layer (Masland and Mills, J. Cell. Biol, 1979; Hayden et al., Science, 1980; Vaney et al., J. Comp. Neurol, 1981).

In an attempt to understand the functional meaning of this peculiar geometry, I have been studying the light-stimulated release of 3H-acetylcholine from isolated retinas incubated in the presence of 3H-choline and then rapidly superfused in a small-volume chamber. When the retina is stimulated by flashing light, the rate of appearance of 3H-ACh in the medium increases, with a latency shorter than the temporal resolution of the system (5 sec.) When the flashing light is turned off, the rate of appearance of 3H-ACh rapidly returns to basal levels. A 1 minute stimulation with steady light causes a burst of ACh release at stimulus onset and a second burst at stimulus cessation. That the bursts represent separate ON and OFF transients -- rather than a resultant of slower, opposing releases -- was shown by experiments carried out in the presence of 2-amino-4-phosphonobutyrate, an agent known to selectively eliminate the transmission of ON responses to the proximal retina: steady light then caused ACh release only at stimulus OFF. Taken together, these results indicate that ACh acts in the retina as a conventional (relatively fast) neurotransmitter, with enhanced release occurring at the moments of light illumination or dimming

There is a substantial release of ACh in the dark: the peak light-evoked release exceeds the dark release by only a factor of 5. Exposure of the retina to medium containing 20 mM Mg++ and 0.2 mM Ca++ entirely prevented the release of ACh evoked by light or by stimulation with large (3 mA) electrical currents, but it depressed the release of ACh in the dark by only 35%. Evidence from the neuromuscular junction (Katz and Miledi, Proc. Roy. Soc. Lond., 1981 suggests that the resudual release may represent non-quantal ACh leakage. This possibility is supported by the finding of Ariel and Daw (J. Physiol., 1982) that some ganglion cells have their spontaneous activity, but not their light evoked response, enhanced by anticholinesterase -- implying a light-independent release of ACh.

It thus appears that the cholinergic amacrine cells release ACh in two ways. The first is rapid and conveys information about changes in illumination from cell to cell. The second may be a non-quantal background leak, unaffected by the functional state of the neuron.

144.2 AFFINITY FOR ^{3}H-DESIPRAMINE BINDING CORRELATES WITH THE POTENCY OF INHIBITION OF ^{3}H-NOREPINEPHRINE UPTAKE BLOCKERS BUT NOT SUBSTRATES OF UPTAKE. S. Arbilla*, M. Sette*, R. Raisman*, M. Briley*, and S.Z. Langer. Dept. of Biology, Synthélabo-LERS, 75013 Paris, France.

^{3}H-desipramine (^{3}H-DMI) binds to specific high-affinity sites in the peripheral and central nervous systems. Lesion experiments in the brain using 6-OH dopamine and in the peripheral nervous system using surgical denervation have shown that high-affinity ^{3}H-DMI binding sites are localised on noradrenergic nerve terminals. As previously reported for the heart and brain, the inhibition of ^{3}H-DMI binding, in the rat vas deferens, by blockers of ^{3}H-norepinephrine (^{3}H-NE) uptake is highly correlated with their potency at inhibiting ^{3}H-NE uptake (Table 1)(correlation coefficient =0.95 n=6). Previous studies have not, however, investigated the ^{3}H-DMI binding and ^{3}H-NE uptake potencies of substrates for the neuronal uptake of ^{3}H-NE. Investigation of ^{3}H-NE uptake substrates such as, dopamine, tyramine, metaraminol and norepinephrine showed, however, that they were much less potent at inhibiting the binding of ^{3}H-DMI than would be predicted from their potency at blocking the uptake of ^{3}H-NE into the vas deferens. This result suggests that although the ^{3}H-DMI binding site is associated with the uptake mechanism for ^{3}H-NE, it would seem that the ^{3}H-DMI binding site and the substrate recognition site for the neuronal uptake of ^{3}H-NE are not identical. It is interesting to note that a similar situation exists for the ^{3}H-imipramine binding site and the uptake of serotonin.

Table 1.	IC_{50} (^{3}H-DMI binding) (μM)	IC_{50} (^{3}H-NE uptake) (μM)
Inhibitors		
Desipramine	0.005	0.037
Nisoxetine	0.008	0.068
Imipramine	0.117	0.713
Cocaine	4.400	4.270
Fluoxetine	4.600	10.110
Substrates		
Dopamine	7.500	1.780
Tyramine	30.000	2.170
Metaraminol	34.500	0.399
(-)Norepinephrine	112.000	2.600

These results suggest that ^{3}H-DMI may bind to the recognition site of a unit that modulates neuronal uptake of NE, rather than to the substrate recognition site of the transporter.

144.3 ATP HYDROLYSIS MEDIATES CALCIUM EFFECTS ON NEURONAL MEMBRANE UPTAKE OF ^{3}H-(-)NOREPINEPHRINE IN RAT CEREBRAL CORTEX. E.D.Hendley Y.H. Ehrlich, S.R. Whittemore* and D.G. Atwater*. Univ. Vermont Coll. Med., Burlington, VT 05405.

The uptake of ^{3}H-(-)norepinephrine was determined in synaptosomal preparations (resuspended P_2) of rat cerebral cortex. These were incubated for 1 min at 30°C, in Krebs-Ringer bicarbonate-buffered medium (KRB) containing EDTA, ascorbic acid and nialamide (10uM). Cocaine, 10 uM, was used to define specific, neuronal membrane accumulation of ^{3}H-(-)norepinephrine at 4 concentrations, from 0.05 to 0.2 uM. Vacuum filtration on Millipore filters (0.45u pore size)was used and the kinetic constants, apparent Km and Vmax were determined. We tested the effects on uptake of removal of either Ca^{++} or Mg^{++}, or both, from whole KRB, and confirmed the findings of T.D. White (J. Neurochem. 24:1037, 1975) using whole brain synaptosomes, that removing both divalent cations from the medium reduced Vmax for uptake of norepinephrine, and that adding back either Ca^{++} or Mg^{++} restored uptake rates to the same levels as with whole KRB. The ability of Mg^{++} (1.2 mM), in the absence of Ca^{++}, to restore uptake rates was not affected by the addition of APP(NH)P, 200 uM, a non-hydrolyzable analog of ATP. In contrast, the ability of Ca^{++} (1.275 mM), in the absence of Mg^{++}, to restore uptake rates was markedly reduced by the addition of APP(NH)P. We concluded that Mg^{++}, in the absence of Ca^{++}, alters uptake by means of an ion effect, whereas Ca^{++}, in the absence of Mg^{++}, appears to require the hydrolysis of ATP. The ID_{50} for APP(NH)P inhibition of this Ca^{++}-induced effect on uptake was approximately 20 uM. Inhibition of uptake by APP(NH)P in Mg-free KRB was similar whether Ca^{++} concentration in the medium was 1.275 or 2.55 mM. Experiments currently in progress are designed to test whether the effects of Ca^{++} described above involve a protein kinase-mediated protein phosphorylation, or an ATPase-mediated hydrolysis of ATP, both of which processes can be blocked by use of APP(NH)P.

144.4 IDENTIFICATION OF A SPECIFIC BINDING SITE FOR RESERPINE ON CHROMAFFIN GRANULE MEMBRANES. James A. Weaver* and Jean D. Deupree. Dept. of Pharmacology, Univ. of Neb. Med. Ctr., Omaha, NE 68105.

Reserpine is a specific, competitive inhibitor of biogenic amine transport into neurosecretory vesicles which suggests the existence of a specific binding site for reserpine on the amine transporter. This binding site for reserpine was identified and characterized by conducting ^{3}H-reserpine binding assays with bovine adrenal chromaffin granule ghosts in 0.3 M sucrose containing 1.0 nM ^{3}H-reserpine (28 Ci/mmole), 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) pH 7.5, 0.1 mM EDTA, 4.0 mM $MgCl_2$ and 4.0 mM ATP at 30°C. Reserpine binding was initiated by addition of chromaffin granule ghosts to this assay medium and terminated, after ^{3}H-reserpine binding reached equilibrium, by filtration. Under these assay conditions reserpine, harmine and norepinephrine produced a dose dependent inhibition of ^{3}H-reserpine binding with potencies identical to those observed for the inhibition of catecholamine transport by each compound. These results suggest that specific binding of reserpine to the catecholamine transporter of chromaffin granules may be analyzed by determining the difference between ^{3}H-reserpine binding in the presence and absence of saturating concentrations of norepinephrine. Further evidence that reserpine was binding to the catecholamine site on the transporter was that a) the Kd (7 ± 1 nM) obtained for catecholamine sensitive reserpine binding was similar to the Ki (2.0 ± 0.3 nM) obtained for inhibition of catecholamine transport by reserpine, b) the time required for specific reserpine binding to reach equilibrium was identical to the time required for maximal inhibition of catecholamine transport by reserpine, and c) the binding site density (18 ± 5 nmoles/mg protein) was in the predicted range. Specific reserpine binding to chromaffin granules was found to be dependent upon the presence of Mg^{++} and ATP and inhibited by an uncoupler of oxidative phosphorylation, carbonylcyanid-p-trifluoromethoxy phenylhydrazone (FCCP). These results suggest that binding of reserpine to the catecholamine site on the biogenic amine transporter of chromaffin granules can be analyzed in vitro and that binding is dependent on the presence of Mg^{++}, ATP and/or an electrical potential across the granule membrane. (This research was supported by NIH, Grant No. 15157.)

144.5 PROPERTIES OF SYNAPTOSOME-STORED ^{3}H-DOPAMINE AND ^{3}H-NOREPINEPHRINE. G.S. Takimoto*, J.D. Stittsworth, Jr. and J.K. Stephens* (SPON: D. Turriff). Department of Biomedical Sciences, University of Illinois College of Medicine at Rockford, Rockford, IL 61107.

Striatal and hypothalamic synaptosomes were prepared from striatal slices incubated in the presence of ^{3}H-dopamine (DA) and hypothalamic slices incubated in the presence of ^{3}H-norepinephrine (NE). Synaptosomes which were prepared from tissue slices incubated in the presence of ^{3}H-DA + nomifensine (striatum) or ^{3}H-NE + desmethylimipramine (hypothalamus) were utilized as blanks and retained approximately 15% of the ^{3}H measured in control samples, suggesting that ^{3}H-DA and ^{3}H-NE were preferentially accumulated by their respective nerve terminals. When the loaded synaptosomes were exposed to 0.1-10μM reserpine for time periods up to 20 minutes, the spontaneous efflux of ^{3}H-DA was greatly accelerated, whereas the spontaneous efflux of ^{3}H-NE was unaffected. Measurement of vesicular and extravesicular stored ^{3}H-NE by employing a hypotonic lysis procedure indicated that ^{3}H-NE did not accumulate in the extravesicular compartment subsequent to treatment with reserpine. However, the accumulation of ^{3}H-DA and ^{3}H-NE by striatal and hypothalamic synaptosomes not subjected to the loading procedure could be prevented in the presence of reserpine suggesting that the vesicular uptake of transmitter in both tissues was equally susceptible to reserpine inhibition. The spontaneous efflux of ^{3}H-DA could also be enhanced in the presence of the DA uptake blocker, nomifensine, whereas ^{3}H-NE efflux was unaffected by treatment with the NE uptake blocker, desmethylimipramine. Tyramine, d-amphetamine and 53mM KCl effectively enhanced ^{3}H-DA release from loaded striatal synaptosomes, however, under similar incubation conditions using loaded hypothalamic synaptosomes, d-amphetamine and 53mM KCl were less effective releasing agents. ^{3}H-DA and ^{3}H-NE retained by the striatal and hypothalamic synaptosomes were probably not associated with storage sites containing newly-synthesized transmitter since the spontaneous efflux of neither ^{3}H-DA nor ^{3}H-NE was affected by the addition of unlabelled L-tyrosine to the incubation medium. The results suggest that ^{3}H-DA retained by striatal synaptosomes is incorporated into storage sites more susceptible to depletion by reserpine, d-amphetamine and 53mM KCl than ^{3}H-NE retained by hypothalamic synaptosomes under similar experimental conditions. (Supported by Illinois Heart Association grant-in-aid N-17 and BRSG grant 58107)

144.6 RELEASE AND METABOLISM OF DOPAMINE IN STRIATAL DIALISATES OF RATS: EFFECT OF NARCOTIC ANALGETICS AND NEUROLEPTICS. G. Di Chiara and A. Imperato*, Institute of Experimental Pharmacology and Toxicology, Chair of Toxicology, University of Cagliari, Cagliari, Italy.

Brain dyalysis in vivo has been developed by Ungerstedt and collaborators as a means to study the release of dopamine (DA) in vivo. We have applied this technique to the study of the release of dopamine (DA) and of its metabolites by analyzing striatal perfusates with reverse-phase HPLC. Rats were anaesthetized with halothane and stereotaxically implanted with a 200 μm capillary (3 x 50 Amicon Vitafiber Unit) inserted transversally through both striata. Ringer was passed through the capillary at a rate of 2 μl per min. Every 10 min the effluent was collected from the other end of the capillary into 5 μl of 1 N perchloric acid and directly injected into a HPLC apparatus equipped with a reverse-phase (Nucleosyl ODS 5 μm) and with an electrochemical detector (BAS). A citrate-acetate buffer with octyl-sulfate as ion paring reagent was used to measure DA while a similar buffer, but without octyl-sulfate, was used to measure DA-metabolites DOPAC and HVA. Basal release of DA ranged from 14 to 32 pgr in 10 min. Morphine produced a biphasic effect on DA release. While doses of 0.1-0.25 mg/kg i.v. increased DA-release by more than 100 % the basal levels, higher doses (1.0 - 20 mg/kg i.v.) decreased it by about 50%. All these effects were blocked or reversed by naloxone (2 mg/kg i.v.). DOPAC and HVA-levels were increased through all the doses tested. Other narcotic analgetics (methadone, dextromoranmide) showed a similar pattern. Apomorphine (0.1 mg/kg) administered at the peak of the morphine effect (0.25 mg/kg s.c.) rapidly dropped DA-release to levels lower than the basal ones. These results suggest that narcotic analgetics exert opposite effects on DA-transmission, stimulating DA-release at low doses and depressing it at higher ones. Classic neuroleptics also had a biphasic effect on DA-release. Thus haloperidol while at higher doses (0.1 - 2 mg/kg i.v.) reduced DA-release up to 80%, at lower doses (0.01-0.025 mg/kg i.v.) increased DA-release. These results indicate that classical indexes of DA-function such as DA synthesis and DA-firing are only indirect since do not necessarily reflect the actual release of DA from pre-synaptic terminals. The present method seems to be adeguate to estimate this fundamental parameter.

144.7 UPTAKE AND PROCESSING OF ANGIOTENSINOGEN BY NEUROBLASTOMA CELLS IN CULTURE. D L Clemens, T. Okamura*, and T. Inagami. Dept. of Biochemistry, Vanderbilt University, Nashville, TN 37232.

All of the components of the renin-angiotensin system have been reported in brain tissue, leading to the concept of an intraneuronal pathway of angiotensin formation. However, study of the pathway in brain tissue is complicated by the inevitable heterogeneity and vascular contamination of brain tissue. Cloned, cultured cells offer obvious advantages of cellular homogeneity and ease of experimental manipulation. We have screened a variety of neuroblastoma cells of rat and mouse origin and found many that contain renin, angiotensin-converting enzyme, and generate angiotensins (Okamura et al, Proc. Natl. Acad. Sci., U.S.A., 79:6940-6943, 1981). When grown in serum free, defined medium, these cells produce renin substrate and release it into the culture medium. Renin substrate is found predominantly in the culture medium and relatively little is found associated with the neuroblastoma cell pellet. Renin, on the other hand, is not secreted into the culture medium, but is found predominantly in intracellular granules. This situation raises the question of whether neuroblastoma cells can use exogenous substrate for the production of angiotensin I. We have found that exogenous renin substrate added to neuroblastoma cells is taken up by the cells and converted to angiotensin I. The uptake mechanism is energy dependent (can be blocked by 2,4 dinitrophenol and 2-deoxyglucose) and shows saturable kinetics. The uptake and conversion of angiotensinogen to angiotensin I is observed only in cell lines with specific renin acitvity, and is not blocked by lysomotropic agents (NH_4Cl, imidazole, or chloroquine). The intracellular site of action of renin in the pathway is substantiated by the failure of specific anti-renin antisera to block the generation of angiotensin by intact neuroblastoma cells, whereas angiotensin generation by lysed cells is totally inhibited by the specific antirenin antisera.

It is concluded that neuroblastoma cells have a capacity for receptor mediated uptake of renin substrate and for specific processing of the prohormone to angiotensin I. The specific uptake and proteoytic processing in neuroblastoma cells may reflect a process with a counterpart in normal neuronal cells.

144.8 COPPER CHELATES: POTENT RELEASERS OF LHRH FROM ISOLATED HYPOTHALAMIC GRANULES. G.E. Rice* and A. Barnea. Green Ctr. Reprod. Sci., Depts. of Ob-Gyn. & Physiol., Univ. of Texas Health Sci. Ctr at Dallas, Dallas, TX 75231.

It has been shown that administration of copper salts stimulates secretion of LHRH from the hypothalamus. We have recently shown that copper, in the form of CuATP, is an extremely potent releaser of LHRH from isolated hypothalamic granules. As ATP appears to be a requirement for the release of other peptides and biogenic amines from secretory granules, we have addressed the question: What is the role of ATP in the copper-stimulated release of LHRH from isolated hypothalamic secretory granules? Granules were prepared from hypothalami of adult male rats and incubated at 37° for 3 min in buffered medium (control), buffered medium containing $CuCl_2$ (0.5mM), or buffered medium containing $CuCl_2$ and one of the following chelating agents: ATP (1mM); tartarate (1.5mM); histidine (1mM); BSA (1mM) or EDTA (0.5mM). At the end of the incubation, LHRH contained in the granules was separated from LHRH released into the medium by gel filtration chromatography. The LHRH recovered in the granule fraction was quantified by RIA. LHRH release (% above control, mean ± SE) was as follows:

	$CuCl_2$	CuATP	CuTartarate	CuHistidine	CuBSA	CuEDTA
Release	13 ± 5	53 ± 5	46 ± 6	11 ± 5	16 ± 2	-2 ± 4

Both CuATP and CuTartarate markedly stimulated LHRH release from the granules and moreover did so with a similar potency. In contrast, CuHistidine, CuBSA, CuEDTA and $CuCl_2$ were ineffective. In addition, we assessed the temperature requirement for the CuATP-stimulated release of LHRH and found that release was 4% from granules incubated at 4°C, 15% at 23°, 13% at 30° and 58% at 37°C. These results are suggestive that a phase-change occurs in the phospholipids of the granule membrane between 30 and 37°C and that such a change facilitates CuATP-stimulated release of LHRH. In summary, we demonstrate that chelates of copper stimulates LHRH release from isolated hypothalamic granules and that this stimulation is related to the equilibrium constants (K_1) of the complexes. LHRH release is stimulated by copper chelates having a $K_1 \leq 10^6$ (CuATP, CuTartarate) but not by those having a $K_1 \geq 10^9$ (CuHistidine, CuBSA, CuEDTA). As it is known that in biological systems copper is present in a chelated form, our results are consistent with the view that chelated copper plays an important role in the release of LHRH from hypothalamic neurons.

144.9 TWO DIFFERENT FORMS OF INACTIVE RENIN IN NEUROBLASTOMA CELLS. T. Inagaki*, D.L. Clemens, and T. Inagami. Dept. of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.

The demonstration of the coexistence of renin and angiotensins in neuroblastoma cells (T. Okamura et al., Proc. Natl. Acad. Sci., 78:6940, 1981, M.C. Fishman et al., Science, 214:921, 1981) suggested the possible intracellular mechanism of angiotensin formation in the central nervous system in contrast to the well known extracellular mechanism in plasma. We have investigated a inactive form of renin in order to clarify a regulatory mechanism of renin activity in the cells. Mouse neuroblastoma cells Neuro 2-a had a high level of renin activity, while rat neuroblastoma cells B103 showed no renin activity in the cell lysate or in culture medium. However, brief treatment of cell lysate of B103 with 1.5 M NaCl/40 mM dithiothreitol was found to cause generation of a large amount of renin activity, whereas Neuro 2-a showed only a minor degree of activation upon the NaCl/dithiothreitol treatment. Sephadex G-200 column chromatography of Neuro 2-a lysate showed that NaCl/dithiothreitol activatable inactive renin was found in fractions corresponding to 110,000 molecular weight. Moreover, octyl-sepharose chromatography of the cell lysate also showed an existence of trypsin activatable prorenin which can be activated by trypsin. The molecular weight of the prorenin was estimated as 55,000 by gel filtration on a calibration column of Sephadex G-200. Furthermore, NaCl/dithiothreitol activatable inactive renin was not activated by trypsin; trypsin activatable prorenin was not activated by NaCl/dithiothreitol. These experiments indicated that NaCl/dithiothreitol activatable inactive renin is different from trypsin activatable prorenin. In a separate experiment, renin inhibitor was separated from Neruo 2-a lysate by anti-renin IgG-Sepharose and renin-agarose affinity chromatography. The finding suggested that NaCl/dithiothreitol activatable inactive renin is renin-inhibitor complex. The present results indicated that neuroblastoma cells have two different systems of regulation of renin activity; renin-inhibitor system and prorenin-renin system.

144.10 IN VIVO RELEASE OF NEUROHYPOPHYSEAL PEPTIDES FROM RAT SPINAL CORD Q.J. Pittman, J. Simpson*, D. Ko* and K. Lederis*, Dept. of Pharmacology & Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta. Canada.

There is now evidence that the neurohypophyseal peptides arginine vasopressin (AVP) and oxytocin may function as neurotransmitters in brain. Immunocytochemical studies have established the presence of immunoreactive AVP or oxytocin in neurons in the hypothalamus and in neuronal processes throughout widespread areas of the brain. In spinal cord, AVP and oxytocin fibers appear to innervate dorsal horn and the intermediolateral cell columns. To further characterize this hypothalamic-spinal projection, we have perfused the spinal cord in vivo and carried out radioimmunoassays for AVP and oxytocin in the perfusate.

In urethane-anesthetized rats, a polyethelene cannula was introduced through the cisterna magna and threaded down the spinal cord in the sub-dural space. Through this cannula a KREBS solution was infused at 30 µl/min. A second, pull cannula was placed at the level of the cisterna magna to withdraw fluid at an identical rate. A plug of petroleum jelly placed in the 4th ventricle prevented contamination of the perfusate by cerebrospinal fluid from more rostral areas. Three 30 min. perfusions were carried out; after the first 30 min. sample was collected, the paraventricular nucleus (PVN) was stimulated through a bipolar electrode for the duration of the second perfusion period. Following termination of the electrical stimulation, a third perfusion was carried out for a final 30 min. period.

Perfusates were assayed for AVP or oxytocin using sensitive radioimmunoassays which show little cross-reactivity for other related peptides. In control perfusates, AVP levels ranged from 0.87 - 22.67 pg/ml; oxytocin levels ranged from 2.3 - 8.5 pg/ml. During stimulation of the PVN, levels of both AVP and oxytocin were elevated to 2.44 times (AVP, n = 6) and 19 times (oxytocin n = 6) control levels. The third perfusate showed reduced levels of these peptides approximating levels seen in the first control perfusates. The increase in the levels of these peptides during stimulation was not likely due to contamination of spinal perfusate by blood-borne peptides. When AVP was infused into the jugular vein at a rate of 0.01 µg/kg/min. for the duration of a 30 min. perfusion, AVP levels of the spinal perfusates were not elevated.

These studies support the existence of a hypothalamic-spinal cord pathway and provide further evidence that the neurohypophyseal peptides may act as transmitters.

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144.11 MEMBRANE DEPOLARIZATION RESULTS IN BOTH SP RELEASE AND DECREASED NET PEPTIDE SYNTHESIS IN SYMPATHETIC NEURONS IN VITRO. J.A. Kessler, J.E. Adler, W.O. Bell* and I.B. Black. Div. of Developmental Neurology, Cornell University Medical College, New York, New York 10021.

Mechanisms regulating the putative peptide neurotransmitters, substance P (SP) and somatostatin (SS), were compared in a variety of neuronal populations in culture. SP increased more than 25-fold within 48 hours in sympathetic neurons in the rat superior cervical ganglion (SCG) in vitro, and peptide levels remained elevated in cultures maintained for 4 weeks. Identity of the peptide was authenticated by combined high pressure liquid chromatography-radioimmunoassay. Veratridine prevented the increase in SP in vitro, and tetrodotoxin blocked the veratridine effect, suggesting that sodium ion influx and membrane depolarization prevent the increase in SP.

To investigate the mechanisms mediating the effects of membrane depolorization, SP release into the culture medium and ganglion peptide content were simultaneously examined after veratridine treatment. Veratridine-(or potassium-) induced membrane depolarization released SP from sympathetic neurons by a calcium-dependent process. Consequently, at least some of the effects of the drug can be attributed to release and subsequent depletion of ganglion peptide. However, the inhibitory effects of veratridine on ganglion peptide content (150 pg less than control) were far greater than could be accounted for by the quantity of peptide released (30 pg), suggesting a separate effect on net synthesis (synthesis less catabolism) of SP. Viewed in conjunction with previous studies in vivo, our observations suggest that trans-synaptic impulses, through the mediation of post-synaptic sodium flux, release SP from sympathetic neurons and also regulate peptide metabolism.

To determine whether the processes regulating SP in sympathetic neurons reflect generalized mechanisms, a different peptide transmitter, SS, was examined in sympathetic neurons, and SP was examined in a different neuronal population, special sensory neurons in the nodose ganglion. Explantation resulted in significant increases in SP in both sympathetic and sensory neurons, and in SS in sympathetic neurons. In each instance the increase was prevented by veratridine, and tetrodotoxin blocked the veratridine effect. Our observations suggest that there may be a group of regulatory mechanisms which govern peptide transmitter metabolism throughout the nervous system. (This work was supported by NIH grants NS 17285, NS 10259, HD 12108 and American Parkinson Disease Association).

144.12 SEQUENTIAL SECRETION (QUEUING) OF COORDINATELY SYNTHESIZED NEUROPEPTIDES. S. Arch and A. Linstedt*. Biological Laboratories, Reed College, Portland, OR 97202.

The bag cell neurons of Aplysia californica synthesize, transport, and secrete a group of polypeptide substances. These molecules may have a common origin in a single translational product. Despite the evidence for coordinate synthesis, we have found that at least two of the posttranslational product peptides are not transported coordinately. After a 6-hr pulse of labeling in ^{3}H-leucine, one of these labeled peptides (AP) appears in transport considerably earlier than the other (ELH). This situation allowed us to examine the organization of the secretory terminal. Since the arrival times at the terminals for the labeled peptides differ, it should be possible to determine if the order of arrival is relevant to access to active secretory sites. The least complex prediction would be that free mixing of vesicles occurs in the terminal and arrival order is not preserved at secretion. In fact, when bag cell organs are challenged with a high $[K^+]_o$ depolarizing medium after both labeled peptides have reached the terminal, only labeled AP (earlier arrival) is secreted in abundance. If a short period of recovery is allowed and a second challenge performed, both labeled peptides are secreted. Thus, free mixing does not appear to occur. Instead, arrival order is maintained as though the vesicles were entering a queue. We have explored the nature of the queuing process by exposing the cells to colchicine or procaine shortly before a first-secretion challenge. Both agents disrupt the order in the queue. Since each is known to affect microtubule organization, there is reason to believe that one element in the constraining structure of the terminal is polymerized and anchored microtubules. While the physiologic significance of secretory queuing is not obvious, its occurrence is entirely consistent with the evidence for extensive fibrous protein structuring in the axonal and terminal cytoplasm of neurons.

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145.1 A PRESYNAPTIC LOCUS OF ACTION FOR TETANUS TOXIN: QUANTAL ANALYSIS OF EFFECTS ON MONOSYNAPTIC TRANSMISSION IN MOUSE SPINAL CORD NEURONS IN CULTURE. G. K. Bergey, H. Bigalke* and P. G. Nelson. Lab. of Developmental Neurobiology, NICHD, Bethesda, MD 20205.

Morphological evidence from intact preparations has demonstrated that, after reaching the ventral spinal cord, tetanus toxin moves trans-synaptically to presynaptic endings on the motoneurons. To date, however, no direct physiologic evidence has been presented to establish a presynaptic locus of action for the toxin in the central nervous system where tetanus produces its major effects. We report here the observed presynaptic effects of tetanus toxin on identified monosynaptic connections between pairs of fetal mouse spinal cord neurons grown in dissociated cell cultures.

Intracellular recordings were made with 4 M KAc-filled microelectrodes using conventional bridge circuits. Cultures used for recordings were 4 to 8 weeks old and were washed free of horse serum and placed in MEM with 1% fetal calf serum. Purified tetanus toxin was applied to yield a final concentration of 100 ng/ml. A computer program for on-line analysis of PSPs was used to determine PSP amplitude and quantal parameters using a modified PDP 11/34 computer. Recordings were done in media containing a final concentration of 6 mM calcium and 6 mM magnesium to reduce spontaneous synaptic activity yet permit evoked synaptic transmission.

After a latent period of about 40 to 60 minutes tetanus toxin (100 ng/ml) produced increased excitation characterized by paroxysmal depolarizing events. Inhibitory synaptic transmission was preferentially reduced by tetanus but excitatory transmission was also later reduced and blocked by the toxin. In these studies quantal analysis was restricted to EPSPs selected so that corrections for nonlinear summation were not necessary. Inhibitory postsynaptic potentials were not used for quantal analysis because saturation was often present due to the proximity of the IPSP reversal potential to the resting membrane potential when KAc electrodes were used.

Quantal analysis (sample time 18 to 195 minutes) was performed on 7 monosynaptic excitatory connections at various times after the onset of convulsant action produced by the toxin. The averaged amplitude of the evoked EPSP was noted to progressively diminish while the number of failures increased. The quantal number diminished parallel to the reduction in EPSP size while the mean quantal size was not reduced. Postsynaptic responses to GABA, glycine and glutamate were not affected by the toxin. These results demonstrate that tetanus toxin acts to reduce presynaptic release of transmitter rather than affecting postsynaptic sensitivity.

145.2 TRANSMITTER RELEASE AS A FUNCTION OF HOLDING POTENTIAL AT THE SYNAPSE OF THE BARNACLE PHOTORECEPTOR. Jon H. Hayashi*, John W. Moore, and Ann E. Stuart, Univ. of North Carolina, Chapel Hill, N.C., and Duke Univ., Durham, N.C.

Input/output relations at the photoreceptor (PR)/ second-order (I-cell) synapse in the giant barnacle were obtained by making simultaneous intracellular recordings from the pre- and postsynaptic cells. The axons of the 4 median PRs travel in the median ocellar nerve (MON) to the supraesophageal ganglion where upon entry they arborize abruptly and contact the I-cells. A PR's axon was impaled just before this arborization (about 1/40 λ from the release sites) and an I-cell was impaled in its soma. The MON was led through an extracellular high-resistance barrier separating two chambers; the PRs' terminal voltages could be altered by passing current between these chambers with bath electrodes. The potential of the impaled PR was fed back to this current-passing circuit in order to generate square voltage steps in the PRs. In separate experiments, simultaneous recordings were made from 2 PRs, one providing feedback and one following. Since waveforms in the 2 PRs were similar in amplitude and rise time, we expected that equivalent control was exerted over all 4 PRs.

Depolarization of the PRs causes them to release a transmitter that hyperpolarizes the I-cell (Stuart & Oertel, Nature 275:287, 1978). When we held the PR voltage at its dark resting potential (-60mV), depolarizations from this level evoked hyperpolarizations in the I-cell that decayed even though the PR's voltage was maintained. Hyperpolarizations of the PR caused a slight (3-5mV), steady depolarization of the I-cell. Thus there is continuous release from this synapse in the dark. To determine the transfer function, the PRs were held 10-20mV negative to the dark resting potential, where release is shut off, then depolarized from this level. The peak responses of the I-cell were plotted as a function of the voltage to which the PR was stepped. Input/output relations rose exponentially, producing a 10-fold change in I-cell voltage for a presynaptic change ranging from 5.4-11.2 mV (n = 7).

When the PR's holding potential was changed to new steady levels, the position of the input/output curve shifted along the PR voltage axis. In addition, the maximal response of the I-cell increased as the PR's holding potential became more negative. This "synaptic adaptation" occurred in less than 0.5 sec of a shift in potential. It was observed over the physiological range of presynaptic potentials. Thus the range of presynaptic voltages over which the PR releases transmitter changes with a PR's steady membrane potential, normally set by the intensity of incident light.

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145.3 **PRESYNAPTIC CALCIUM DIFFUSION AND TRANSMISSION AT THE SQUID GIANT SYNAPSE.** R.S. Zucker and N. Stockbridge*, Physiology-Anatomy Dept., Univ. of Calif., Berkeley, CA 94720 and Physiology Dept., Duke Univ., Durham, N.C. 27710.

We have applied a mathematical model for intracellular calcium movements, developed initially for the frog neuromuscular junction, to explain a number of observations obtained at the squid giant synapse (Charlton *et al.*, 1982). A presynaptic action potential is accompanied by an influx of calcium ions that continue to be detectable with arsenazo III microspectrophotometry for several seconds. Nevertheless, transmitter release occurs phasically, lasting only about 2 msec. A second action potential is accompanied by an identical calcium influx, but transmitter release is increased (facilitated) if the action potentials are separated by 50 msec or less.

The model is described in an abstract by N. Stockbridge and J.W. Moore. The presynaptic terminal is represented as a long cylinder, 50 μm in diameter. Calcium influx during an action potential was represented as a square pulse with amplitude and duration equal to that estimated by Llinas *et al.*, (1981). Intracellular calcium diffused radially with 97.5% of the total calcium rapidly binding to fixed cytoplasmic sites (Brinley *et al.*, 1977; Baker & Schlaepfer, 1978). Calcium was removed from the terminal by a surface pump at the rate of 2×10^{-4} cm/sec (Dipolo, 1976). Mitochondrial buffering of weak calcium loads is negligible (Requena & Mullins, 1979) and was ignored. The temporal kinetics of endoplasmic reticulum buffering are yet to be determined (Blaustein *et al.*, 1978) and have not been included. Transmitter release was taken as proportional to the square of the submembrane calcium concentration (Charlton *et al.*, 1982).

Equations representing these processes were solved for two action potentials separated by different intervals, with the following results: 1) A brief release of transmitter, dropping to 10% in 2 msec, followed each action potential. 2) The second action potential released more transmitter than the first, with facilitation decaying with a rapid phase lasting about 5 msec and a slower phase lasting about 50 msec. 3) The total free calcium in the terminal was removed very slowly, with a time constant of about 5 sec. These predictions match experimental results.

The behavior of this model for squid synapses is similar to its behavior at frog neuromuscular junctions, except that facilitation is briefer in squid. This is due to the more rapid diffusion of calcium into the large central core of the giant squid terminal, and the lower level of calcium binding used in this simulation. The dramatic differences in time scale between transmitter release and facilitation on the one hand and calcium removal from the terminal on the other are due to the fact that the former processes are limited by the rapid diffusion of calcium away from transmitter release sites while the latter is limited by the calcium extrusion pump.

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145.4 ACTH CAUSES LONG-LASTING POTENTIATION OF TRANSMITTER RELEASE FROM FROG MOTOR NERVE TERMINALS. M. F. Johnston*, E. A. Kravitz, R. Rahamimoff, and H. Meiri. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115, and Dept. of Physiology, Hebrew Univ.-Hadassah Med. Sch., Jerusalem, Israel.

Recent reports of low transmission safety factor at some motor endplates (Grinnell and Herrera, J. Physiol. 307: 301) suggest that a capability for increasing transmitter release might be important for muscle function under normal conditions where synaptic depression is likely. Use-dependent effects such as facilitation and potentiation act and subside quickly, while longer-term modulation can be induced by exogenous agents such as catecholamines (Kuba, J. Physiol. 211: 551) and Substance P (Steinacker, Nature, 267: 268).

We report here that adrenocorticotrophic hormone (ACTH) and related peptides can produce long-lasting potentiation of both evoked and spontaneous transmitter release in cutaneous pectoris and sartorius preparations. Following a 15-30 minute bath application of 0.5-3 μM bovine ACTH, α-melanocyte stimulating hormone, or synthetic ACTH(1-24), endplate potential (EPP) amplitude and miniature endplate potential (MEPP) frequency increase up to two-fold over control levels and persist for as long as the penetration remains stable (up to five hours). The preparation does not respond further to subsequent trials of hormone. MEPP amplitude remains constant, so the EPP effect reflects a rise in quantal content. Synthetic ACTH(4-10) is ineffective.

ACTH-induced increases in EPP amplitude and MEPP frequency are seen in the presence of normal Ca^{++} or low Ca^{++}/high Mg^{++}. MEPP frequency also increases in response to ACTH in solutions with no added Ca^{++}.

Hormone treatment produces no changes in muscle fiber resting potential or tension. The rise in EPP quantal content and MEPP frequency in the face of constant MEPP amplitudes argues strongly for a presynaptic site of action for these peptides.

Since these hormone actions do not depend on the level of extracellular Ca^{++}, we doubt that mechanisms involving only an increase in the terminal's surface membrane Ca^{++} permeability can explain these results.

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145.5 TEMPORAL FACILITATION OF GRADED ANTIDROMIC POTENTIALS IN AN ELECTROTONICALLY COUPLED NUCLEUS: A MODEL FOR FACILITATION AT ELECTROTONIC SYNAPSES. Michael V.L. Bennett and George Pappas. Dept. Neuroscience, Albert Einstein Col. Med., Bronx, NY 10461, & Dept. Anatomy, U. Illinois, Coll. Med., Chicago, IL.

The electromotor neurons of the stargazer, Astroscopus, are electrotonically coupled as shown by direct measurement, and gap junctions are plentiful in the electromotor nucleus. Graded antidromic stimulation produces graded depolarizations that decay with a time constant of about 50 msec. Gradedness arises from activation of additional axons whose impulses spread to the cell whose potential is being recorded. The slow time course results from passive recharging of the membrane capacity according to its time constant. In many cells antidromic invasion fails at the initial segment or first node. Increase in the antidromic volley can facilitate invasion because of depolarization spread from adjacent cells. Depolarization remaining from a previous antidromic volley can also facilitate invasion. For this reason,the graded antidromic depolarizations show pronounced facilitation. They also show summation and, at short intervals, depression. Facilitation occurs with both paired and tetanic stimulation and can more than double the size of a test response. During a tetanus the peak depolarization can be increased 5 fold over the initial response because of the combined effect of summation and facilitation. In this system increase in response amplitude results from increase in amplitude of what is in effect the presynaptic potential. The duration of facilitation is determined by the passive membrane time constant. Increase in the duration of the presynaptic impulse could also facilitate the response at an electrotonic synapse and the time course could then well outlast the membrane time constant. However, calculations show that the degree of facilitation is greater if the membrane time constant is long compared to the impulse duration. These results provide a model for temporal effects in electrotonically coupled systems such as the dendrodendritic networks in mammalian CNS. Regions of low safety factor for conduction, changes in impulse properties and long membrane time constants could interact to allow temporal effects over intervals long compared to the propagated impulse and to mediate neuronal processes considerably more subtle than precise synchronization of firing.

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145.6 FACILITATORY TRANSMITTER INCREASES THE CONTENT OF cAMP IN SINGLE NEURONS OF APLYSIA. Lise Bernier*, Vincent F. Castellucci, Eric R. Kandel, and James H. Schwartz. Center for Neurobiology and Behavior, College of Physicians and Surgeons of Columbia University, and New York State Psychiatric Institute, New York, N. Y. 10032.

Short-term sensitization of the gill and siphon-withdrawal reflex in the marine mollusk Aplysia is a simple form of learning that lasts for 20 min to an hour. Underlying the behavioral change is a cascade of biochemical events that leads to enhanced release of transmitter. The first step in this cascade was postulated to be an increase in cAMP within sensory neurons. We have developed labeling protocol with ^{32}Pi which permits us to measure reliably the synthesis of cAMP in single neurons. We have found that application for 5 min of 0.2 mM serotonin, the putative facilitatory transmitter, increased the cAMP content of individual sensory neurons 3-fold. The response is specific to serotonin: dopamine, a transmitter that does not produce sensitization, did not increase cAMP in these cells. Physiological stimulation of the facilitator neurons which results in release of the endogenous facilitatory transmitter also increased the content of cAMP in sensory cells 3.5-fold, but did not affect other neurons of the ganglion. We studied the time course of the increase in cAMP in sensory cells stimulated with serotonin, and found that it parallels very closely the time course of the short-term form of presynaptic facilitation. Short-term sensitization can be converted to the long-term form lasting for weeks by repeated training. In animals trained for long-term sensitization, the amounts of cAMP in sensory neurons was found to be the same as in naive controls, suggesting that a persistent elevation in the cyclic nucleotide is not the molecular basis of long-term memory.

Sensory neurons in the pleural ganglion also mediate sensitization, and they too show a serotonin-sensitive adenylate cyclase (Pollock, Camardo, Bernier, Schwartz and Kandel, these abstracts). Thus, this kind of cyclase may be an important component in mediating a general class of synaptic plasticity involved in learning.

145.7 PLEURAL SENSORY NEURONS OF APLYSIA: A NEW PREPARATION FOR STUDYING THE BIOCHEMISTRY AND BIOPHYSICS OF SEROTONIN MODULATION OF K^+ CURRENTS. J. D. Pollock*, J. S. Camardo*, L. Bernier*, J. H. Schwartz, and E. R. Kandel (SPON: Claude Ghez). Center for Neurobiol. & Behav., P & S, Columbia Univ., and N.Y.S. Psychiatric Institute, N. Y., N. Y. 10032.

Sensory neurons in the abdominal ganglion of Aplysia show presynaptic facilitation in response to stimulation of interneurons thought to be serotonergic. 5-HT decreases a novel K^+ current by a cAMP-dependent phosphorylation (Camardo et al., Neurosci. Abstr., 7, 836, 1981). Protein phosphorylation leads to an increase in the duration of the action potential, prolongs Ca^{++} influx, and enhances transmitter release. These events underlie sensitization of the gill-withdrawal reflex. The two sensory clusters in the abdominal ganglion that innervate the siphon and mantle shelf each contain approximately 24 cells that are about 40 μm in diameter. The small number of cells and their size limit biochemical studies. We therefore have now examined two symmetrical clusters of sensory neurons in the paired pleural ganglia with receptive fields in the tail and body wall (Walters et al., Neurosci. Abstr., 7, 353, 1981). Each cluster contains about 200 similar cells that are on average 60 μm in diameter. These neurons also show presynaptic facilitation (Walters et al., 1981) and 5-HT delays repolarization of their action potential. Since the availability of these clusters would greatly aid future molecular studies of sensitization, we sought to determine whether the current modulated by 5-HT in the pleural sensory cells resembles that of the abdominal sensory cells in its biophysical properties and its dependence in cAMP.

We first analyzed the action of 5-HT on the pleural cells by voltage clamp. The 5-HT-modulated outward current can be detected within 5 msec of depolarization from -50 to 0 mV, and shows no inactivation after sustained depolarization. The decrease is unaffected when Ba^{++} is substituted for Ca^{++}. Intracellular injection of cAMP simulates the action of 5-HT. In addition, application of 5-HT stimulates the synthesis of cAMP to an extent (3.7X) similar to that found in sensory neurons of the abdominal ganglion (Bernier et al., 1982).

We conclude that 5-HT increases the activity of an adenylate cyclase in these pleural cells and that 5-HT and cAMP decrease a net outward current with properties similar to the serotonin-sensitive K^+ current in the abdominal cells. These findings suggest that the same molecular mechanism for presynaptic facilitation operates in the two sensory systems. That a mechanism involving cAMP and protein phosphorylation underlies sensitization in these pleural cells is of further interest because their larger size and number will permit a detailed biochemical analysis of the intracellular events that accompany elevation of cAMP by 5-HT.

145.8 DECREASE IN INTRACELLULAR CA ACCOMPANYING A SEROTONIN-INDUCED REDUCTION OF OUTWARD CURRENT IN IDENTIFIED NEURONS OF HELIX ASPERSA. M.B. Boyle and S.J. Smith*. Department of Physiology, Yale University School of Medicine, New Haven, CT 06510.

A serotonin-induced reduction in net outward current depending both on voltage and on extracellular Ca has been described in identified molluscan neurons. Different investigators have attributed the outward current reduction either to increases in inward Ca current or decreases in outward K current. To further define the role of Ca in this serotonin (5HT) response, we have used Arsenazo III to measure intracellular free Ca in the identified cells of Helix previously studied by Paupardin-Tritsch et. al. (Br. Res. 217:201-206).

Optical absorbance of dye-filled cell bodies was measured simultaneously at 570, 610, 660, and 700 nm. The bath contained 10 mM Ca. Two voltage-clamp protocols were used. In the first, cells were steadily depolarized to between -20 and +20 mV. During such depolarizations, sustained increases in intracellular Ca were observed. In the second, cells were depolarized once per minute from -40 mV through a sequence of steps lasting a few seconds to voltages between -30 and -10 mV. Each pulse sequence led to a transient increase of intracellular Ca, and recovery back to baseline occurred with repolarization to the holding potential. 5HT was applied iontophoretically. When 5HT was applied during steady depolarization, Ca concentration was seen to decline and then to recover along a time course similar to that of the current response. If 5HT was applied during repetitive depolarizing pulse sequences, the baseline Ca also declined. In addition, the depolarization-induced Ca transients were reduced in a graded manner corresponding in magnitude and time course to the onset and recovery of the current change.

We conclude that: 1) the inward current response is not due to an increased Ca current; instead, the 5HT effect is accompanied either by a reduction in inward Ca current or stimulation of Ca removal from cytosol; 2) the decreases in intracellular Ca could themselves account for the decreased outward current, by reducing activation of Ca-dependent K current. Cyclic AMP has previously been implicated in the mechanism of the 5HT inward current response, and reduction of intracellular Ca by 5HT is therefore reminiscent of the cAMP-mediated stimulation of Ca removal in heart by epinephrine. Suppression of intracellular Ca transients by 5HT during voltage-clamp pulses raises the possibility that 5HT-induced increases in action potential duration could be associated with decreases rather than increases in Ca accumulation during action potentials. It will be important to resolve this issue since increased Ca entry during action potentials has been proposed as a mechanism for increased transmitter release in heterosynaptic facilitation. Supported by NIH Grant NS16671.

145.9 STIMULATION OF ADENYLATE CYCLASE IS NECESSARY FOR 5HT ACTIVATION OF A K+ CHANNEL IN APLYSIA NEURON R15.
José R. Lemos* and Irwin B. Levitan (SPON:B.Gähwiler) Friedrich Miescher-Institut, P.O.Box 273,CH 4002-Basel, Switzerland.

Serotonin (5HT) causes an increase in K+ conductance in Aplysia neuron R15. A great deal of evidence suggests that the activation of this K+ channel is mediated by the stimulation of an adenylate cyclase and a consequent increase of intracellular cAMP (Drummond, et al.,1980). In order to test whether cyclase stimulation is a necessary step in the sequence of events that leads to the increase in K+ conductance, we injected a GDP analog, GDPβS, into cell R15 and measured its response to 5HT. GDPβS has previously been shown to inhibit many cyclase systems in vitro (Cassel, et al. 1980), apparently by competing for the GTP binding site on the regulatory subunit of adenylate cyclase, and we have found that it also inhibits 5HT-stimulated cyclase in membranes from Aplysia nervous system.

Following its pressure injection into voltage-clamped R15's, GDPβS has no apparent effects on electrical activity. However, within minutes, the response of R15 to 5HT is markedly altered. If low concentrations (μM intracellular) of GDPβS are used, the 5HT activation of K+ conductance is totally blocked. This effect persists for many hours. On the other hand, the cell's response to dopamine is unaffected by GDPβS injection. (Dopamine affects a different conductance than 5HT (Wilson and Wachtel, 1978) and its action is not mediated by cAMP). Ten to 24 hours after GDPβS injection the cell can again respond to 5HT in a normal manner. Thus the stimulation of adenylate cyclase appears to be a necessary step in 5HT activation of a K+ channel in cell R15.

In contrast, much higher concentrations (mM intracellular) of GDPβS produce quite different results in R15. Direct effects on membrane conductance are observed after 1-2 hours. In addition the response to 5HT is markedly enhanced: large increases in K+ conductance, which cannot be reversed by washing, are observed at 5HT concentrations as low as 10 nM. GDPβS has no detectable effects on Aplysia phosphodiesterase or protein kinase activities, or on serotonin binding. The mechanism of action of GDPβS at high concentrations is being investigated.

145.10 CHLORIDE CHANNELS OPENED BY CHOLINERGIC AGONISTS IN SNAIL NEURONS.
P. Ascher* and S.D. Erulkar[1]. Laboratoire de Neurobiologie, Ecole Normale Supérieure, 75005 Paris, France.

The elementary properties of cholinergic Cl^- channels of snail neurons have been analyzed using the various configurations of the patch-clamp technique (Hamill, O.P. et al., Pflügers Arch., 391:85, 1981). After incubation of the isolated oesophageal ganglion in trypsin (TPCK Worthington, 1 mg/ml, 30 min at 32 °C), the connective tissue sheath was removed and the neurons gently agitated until the somas separated from the axons. The isolated cell bodies were transferred to Falcon culture dishes on which they adhered. In order to identify the Cl channels, we relied on the fact that suberyldicholine (SUB) is a selective agonist of the Cl system and does not activate either excitatory channels or the inhibitory K^+ conductance (Ger, B.A. and Zeimal, E.V., Brain Res., 121:131, 1976 ; Kehoe, J.S., Adv. Pharmacol. Therap., 8:285, 1979). On "outside-out" patches, a prolonged activation of Cl^- channels was obtained with SUB 25 nM or ACh 250 nM or Carbachol 1000 nM. At higher concentrations the receptors appeared to become desensitized and shortly after drug administration, were no longer activated.

The majority of the records showed channel openings with a single conductance state of 15 ± 2 pS, the value being similar for all three agonists. However, in some records, "partial" conductances were observed similar to those described by Hamill and Sakmann (Nature, 294:462, 1981) and Trautmann (Nature, in press). In addition, with ACh and Carb, but not with SUB, some "outside-out" patches showed excitatory cationic channels as well as Cl selective channels. Both types of channels had similar conductances, but the reversal potential for the Cl^- channel was 0, and that for the excitatory channels, - 20 mV (the composition of the "internal" solution was (mM) : KCl, 75 ; EGTA, 5.5 ; $CaCl_2$, 0.5 ; Tris Cl, 10 ; that of the external solution was (mM) : NaCl, 80 ; KCl, 4 ; $CaCl_2$, 10 ; $MgCl_2$, 5 ; Hepes, 5 ; Glucose, 10).

Neither the histograms of open times for outside-out patches nor the noise spectra obtained in the "whole-cell" configuration could be fit to a single time constant (cf. Gardner, D. and Stevens, C.F. J. Physiol., 304:145, 1980). A reasonable fit was obtained with two time constants ; for equiactive concentrations the value of the slow time constant varied in the order $\tau_{Carb} < \tau_{ACh} < \tau_{Sub}$ as described at the frog neuromuscular junction.

In contrast to Cl^- channels obtained in "outside-out" patches, Cl^- channels could only be obtained rarely with the "cell-attached" configurations (25 nM SUB present in the electrodes). This is tentatively attributed to the difference in the surface of the membrane analyzed with the same pipette in the two configurations.

[1]Senior Macy Scholar ; partial support from NS12211.

145.11 DESENSITIZATION OF GABA RECEPTOR OBSERVED IN APLYSIA.
Mitsuhiko Matsumoto*, Kohichiro Takashima*, Masanori Shozushima* and Makoto Sato. Dept. Physiol., Sch. Med., Iwate Med. Univ., Morioka, 020 JAPAN.

Many cells in R_B group of the abdominal ganglion are hyperpolarized by a topical application of GABA. The hyperpolarization is associated with a characteristic, rapid increase in membrane conductance toward Cl^-. This type of receptor activity was evaluated by GABA-induced current and conductance change measured under voltage clamp of the resting membrane.

The response to a sustained application of GABA was characterized by an initial rapid increase in conductance followed by an exponential decay to the original value despite the presence of GABA. This decay of the response was found to be largely due to the receptor desensitization.

The rate of desensitization (α) increased when the concentration of GABA increased, showing a sigmoidal curve in α-log[GABA] relation. This relationship suggested that a binding of another GABA molecule to the activated receptor induces the state of desensitization.

The binding of GABA to the desensitizing site was found independent to the voltage across the receptor membrane, whereas the binding to the activating site of the same receptor showed a characteristic voltage dependence. This observation suggested a functional or structural difference between two binding sites in the same receptor moiety.

On the basis of these results, we propose an allosteric model that the activated receptor-GABA complex makes additional binding with a GABA molecule in order to be desensitized.

145.12 RUTHENIUM RED ENHANCES DESENSITIZATION OF ACETYLCHOLINE RECEPTORS.
Angeles B. Ribera and William L. Nastuk. Dept. of Physiology, Columbia Univ., NY, NY, 10032.

Calcium ion (Ca^{++}) influx increases across the agonist-depolarized postjunctional membrane (PJM). Nastuk and Parsons (J Gen Physiol 56:218, 1970) postulated that desensitization (DS) of the acetylcholine receptor involves the accumulation of Ca^{++} at the inner surface of the PJM. On this basis, inhibition of mitochondrial or sarcoplasmic reticular Ca^{++} sequestration would produce an increase in the intracellular Ca^{++} level and hence enhance DS. We tested this prediction by applying Ruthenium red (RuR) to block mitochondrial Ca^{++} transport in frog sciatic nerve-sartorius muscle preparations.

DS was assayed by measuring the decline in transient depolarizations of the PJM during repetitive iontophoretic application of carbachol (carb). The standard test consisted of a 1 Hz 45 sec train of 10 msec duration carb pulses. A DS level of less than 10% was obtained in control experiments with the preparation bathing in Ringer solution containing 1.8 mM Ca^{++}. After muscle preparations were treated with 1 μM RuR, the standard test produced a 28% DS level. In these RuR treated preparations, the intensification of DS was evident even when the carb train consisted of 5 msec pulses. Under these conditions the standard test produced a DS level of 24%. After RuR treatment the PJM desensitized to repetitively applied carb with a time course similar to that observed in the presence of high (10 mM) extracellular Ca^{++}.

In another series of experiments, the effect of RuR on DS was enhanced by elevating the extracellular Ca^{++} concentration to 10 mM: in RuR treated preparations the neuromuscular junction was perfused with Ringer solution containing 10 mM Ca^{++}. Under these conditions, the standard test produced a 53% DS level.

In addition we found that RuR treatment led to a reduction in the PJM sensitivity. Three different methods were used to determine the PJM sensitivity: (1)iontophoresis of carb, (2)miniature end plate potential amplitude, and (3)microperfusion of carb. By all three tests, the PJM sensitivity was found to be reduced 50%.

We also found that, following RuR treatment, nerve evoked neuromuscular transmission was blocked. RuR may block presynaptic Ca^{++} channels and hence reduce the quantal content (Alnaes and Rahamimoff, J Physiol 248:285, 1975).

These results add further support to the Nastuk-Parsons intracellular model of DS. In addition, the finding that the PJM sensitivity was reduced after RuR treatment raises the interesting possibility that, prior to the application of carb, the PJM was partially desensitized by the RuR treatment. Alternatively, RuR itself may act directly on the PJM to reduce the sensitivity to carb.

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146.1 REGIONAL AND SPECIES COMPARISON OF BRAIN α_2-RECEPTOR STATES. D.C. U'Prichard and B.D. Perry Dept. of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611

The brain α_2-adrenergic receptor (R) exists in two states, differentiated by high (α_2H) or low (α_2L) affinity for agonists. Agonist (^{3}H-epinephrine, ^{3}H-EPI) and partial agonist (^{3}H-p-aminoclonidine, ^{3}H-PAC) radioligands, at low concentrations, selectively label the α_2H state. Antagonists also have demonstrated heterogeneous interactions with the α_2-R, though in a manner reciprocal to agonists. In competition (Ki of rauwolscine, RAUW, vs ^{3}H-EPI 15 nM and vs ^{3}H-RAUW 1 nM, Perry and U'Prichard, Eur. J. Pharmacol. 76:461) and saturation studies (biphasic ^{3}H-RAUW Scatchard where K_D α_2L=1.0 nM, K_D α_2H=40 nM), antagonists have higher affinities for the α_2L state such that ^{3}H-RAUW, at low concentrations, will selectively bind to the α_2L state. Guanine nucleotides and Na^+ will shift the equilibria between the α_2L and α_2H states in favor of α_2L, which is reflected by reciprocal changes in ^{3}H-PAC (↑) and H-RAUW (↑) binding. We have used ^{3}H-PAC (α_2H-selective) and ^{3}H-RAUW (α_2L-selective) to measure relative α_2-R state densities and α_2L/α_2H ratios in 30 bovine, 10 rat and 30 human brain regions, and, in selected regions, have further examined the pharmacology of these α_2-R states in an attempt to analyze regional and species differences in α_2L/α_2H equilibria. Sample values of single point ^{3}H-RAUW binding (Na-K-PO_4 buffer) follow, (% frontal cortex binding): BOVINE (Bmax f.ctx.=160 fmol/mg-prot) caudate:120, cingulate gyrus:115, ant. hypothal.:100, cerebellum:80, sup. colliculus:80, putamen:70, post. vent. medulla: 41, amygdala:40, dorsal pons:14 RAT (Bmax f.ctx.=140 fmol/mg-prot): caud:110, hypothal:96, pons/med:70, cbl:50 HUMAN (Bmax f.ctx.=150 fmol/mg-prot) caud: 80. Sample values for ^{3}H-PAC (Tris HCl buffer): BOV (Bmax f.ctx.=110) caud:40, cing gyr:100, ant. hypo:106, cbl:68, put:50, sup. coll:29, amyg:99, post vent med:24, dorsal pons:12, RAT: (Bmax f.ctx.=140) caud:80, hypothal:90, pons/med:88, cbl:32, HUMAN (Bmax f.ctx.=60) caudate:85. The ratio, % ^{3}H-RAUW f.ctx bind./% ^{3}H-PAC f.ctx binding, was variable across regions within a species (BOV: caud=2.8, amyg=0.4) and across species within a region (BOV: caud=2.8, HUMAN: caud=1.0). Na^+ increased the ratio in all regions (compared to f.ctx values without Na^+). K_D values for ^{3}H-RAUW were lower in bov caud than in other bov regions with an α_2L/α_2H ratio near 1.0 (K_D caud=1 nM, K_D ctx, cbl=2.6) and somewhat higher (K_D= 4.0 nM) in the amygdala (ratio, 0.4). Agonist Ki values in the caud were higher than in regions with a ratio of 1 (Ki, nM: EPI; caud=280 ctx=170, cbl=150: PAC; caud=8, ctx=5, cbl=5) and antagonist Ki values were lower (Ki, nM: YOH; caud=2, ctx=8, cbl=7). The results demostrate that both regional and species differences exist for the α_2L/α_2H equilibria and further demonstrate the need for careful characterization of a given α_2-R system before the effects of experimental manipulation (chronic drug, stress) can be correctly interpreted. (Supported by NS 15595 and NIH Training Grant GM-07263)

146.2 GENETIC ANALYSIS OF AN INVERSE RELATIONSHIP BETWEEN BRAINSTEM α_2-ADRENERGIC RECEPTORS, AND PNMT ACTIVITY AND EPINEPHRINE LEVELS IN RATS. B.D. Perry, J.M. Stolk*, G. Vantini*, J. Hurst*, and D.C. U'Prichard. (SPON: E.M. Silinsky) Dept. of Pharmacology, Northwestern Univ. Medical School, Chicago, IL 60611 and Neuroscience Program, Maryland Psychiatric Research Center, Baltimore MD 21228

There is substantial evidence that genetic factors influence the activity of adrenal and brain catecholamine biosynthetic enzymes. Of particular interest is the relationship, in inbred strains, of adrenal and brainstem phenylethanolamine-N-methyltransferase (PNMT) activity and the levels of brain epinephrine (EPI) formed in the brainstem as a result of PNMT activity. We wished to compare these parameters with the levels of brainstem α_2-receptors (α_2-R) since brain EPI is thought to exert many actions, specifically with regard to control of blood pressure, by activating α_2-R. Breeding colonies of F344 and BUF rats were established from parental stock obtained from Microbiol. Assoc. (Bethesda MD) and hybridization was commenced after two generations of in-house parental strain brother-sister mating. Adrenal and brainstem PNMT were assayed by the methods of Axelrod (J.Biol.Chem. 237, 1657, 1962) and Pendleton (J.Pharmacol.Exp.Ther. 208, 24, 1979), respectively. Brainstem EPI was assayed by HPLC-ECD. α_2-R in washed brainstem membranes were assayed using the agonist, ^{3}H-p-aminoclonidine (PAC), which selectively labels α_2H states, and the antagonist, ^{3}H-rauwolscine (RAUW), which has some selectivity for α_2L states. PNMT and EPI data for male rats are shown below.

Group	N	Adrenal PNMT	Brain PNMT	Brain EPI
F344	23	6.12	0.262	10.77
BUF	36	1.42	0.232	6.46
F344xBUF (F_1)	57	3.86	0.243	9.07

PNMT is expressed as U/tissue (U=1.0 nmol product/hr, 37°) and EPI as ng/tissue. Similar differences were observed in adrenal PNMT for females. In a limited sampling of the above tissue (N=6-12, for each group: males) both brainstem RAUW and PAC binding was 2-3 fold lower in F344 than in BUF, and values for F_1 hybrid were intermediate. For all parameters examined, the differences between F344 and BUF values were significant at at least $P < 0.01$. In females, medullary α_2-R binding was more markedly lower (4-5 fold) in F344 than in BUF (N=5). The results indicate that marked differences between F344 and BUF rats for adrenal PNMT are reflected in similar, but smaller, brainstem PNMT differences and substantial differences in EPI levels. Brainstem α_2-R appear to co-vary in an inverse manner with PNMT and EPI, suggesting that, in the brainstem, EPI content may be a prime regulating factor of α_2R density. The genetic differences are quite specific since, at present, we have observed no significant differences between F344 and BUF for brainstem β- and opiate receptors, and norepinephrine and dopamine content. (Supported by USPHS MH 32842 and NS 15595)

146.3 REGULATION OF RAT CEREBRAL CORTEX α_2-RECEPTOR AFFINITY STATES AFTER ELECTROCONVULSIVE SHOCK AND ANTIDEPRESSANT TREATMENT. A. Garcia*, C.H. Wang, A.I. Salama and D.C. U'Prichard. Dept. of Pharmacology, Northwestern Univ. Sch. Med., Chicago, IL 60611, and Dept. of Biomed. Res., ICI Americas Inc., Wilmington, DE 19897.

Chronic electroconvulsive shock (ECS) and antidepressant (AD) treatments down-regulate β-adrenergic receptors (β-R). Some ADs also cause functional α_2-receptor (α_2-R) subsensitivity, but are reported to cause both increases and decreases in rat brain α_2-R sites labeled with ^{3}H-agonists (^{3}H-clonidine, CLO and ^{3}H-p-aminoclonidine, PAC) which selectively label high affinity α_2(H) receptor states. We have recently characterized binding of the α_2-antagonist ^{3}H-rauwolscine (RAUW) to cortex membranes (Perry and U'Prichard, Eur. J. Pharm. 76, 461, 1981). RAUW labels both α_2(H) and α_2(L) states. Studies were performed to determine whether increases in cortex CLO and PAC sites after AD treatment represented increases in the total α_2-R population, and whether ECS treatment also regulated α_2-R. ECS (100 mA, 300 ms) was administered via ear clips once daily for 1-7 days. All rats receiving ECS experienced generalized tonic-clonic seizures. Animals were sacrificed 18 hr after 1-6 ECS, and 2, 3 or 6 days after 7 ECS. Thawed, washed cortex membranes were assayed for β-R (0.4 and 1.2 nM ^{3}H-dihydroalprenolol, DHA), and for α_2-R using CLO (0.2-16 nM), PAC (0.2-2.5 nM) and RAUW (0.8-8.5 nM). In all α_2-R studies, Kd and Bmax values were determined using 5 ligand concentrations. No Kd change was observed with treatments. ECS significantly reduced DHA binding at each time over 6 days treatment, with a maximum effect (-22%) at day 4. Recovery to control levels began during the daily ECS schedule (day 5), and β-R were no different from control at 6 days after the 7th ECS. Generally, ECS caused an increase in α_2-R binding which returned to control during the ECS schedule. However the time courses of Bmax increases (20-50%) differed for each ligand: the increase in CLO was apparent at day 2, peaked at day 3 and was no longer seen at day 4; increases in PAC and RAUW Bmax did not appear until days 3 or 4, peaked at day 5, and returned to control by 2 days after the 7th ECS. The results suggest that chronic ECS treatment may have sequential effects on cortex α_2-R: (1) an initial subsensitivity seen as the increase in CLO Bmax with no change in RAUW at days 2-3; (2) a subsequent supersensitivity seen as the increase in total α_2-R measured with RAUW. The proposed α_2-R supersensitivity coincides with, and may result in, the the onset of return of β-R to normal levels at day 5 ECS. In preliminary experiments, rats given imipramine (10 mg/kg bid) after 4 weeks showed an increase in cortex CLO Bmax with no change in RAUW Bmax, suggesting that AD treatment does not cause α_2-R supersensitivity but may cause subsensitivity. (supported in part by USPHS grant NS 15595)

146.4 CHANGE OF BETA-ADRENERGIC RECEPTOR NUMBER IN CEREBRAL MICROVESSELS OF SPONTANEOUSLY AND DOCA-SALT HYPERTENSIVE RATS. M. Trabucchi, H. Kobayashi*, S. Magnoni*, A. Cazzaniga*, P.F. Spano* Dept. of Pharmacology and Therapeutics University of Brescia, Italy.

It has been extensively recognized that central and peripheral catecholaminergic neurons play an important role in the regulation of blood pressure. Changes in synthesis, content and turnover of catecholamines are reported in several brain regions, sympathetic ganglia and peripheral tissues of spontaneously (SHR) and DOCA-salt hypertensive rats. Various experimental observations suggest that microvasculature function is altered in hypertension. In fact, morphological studies show the presence of brain capillary injuries in experimental hypertensive monkeys and in human patients affected by essential hypertension. Biochemical evidences suggest that blood-brain barrier (BBB) permeability is increased in SHR. In addition, a diminished response of brain microvessel adenylate cyclase to catecholamines has been reported in SHR.

On the basis of these observations, β-adrenergic receptor function has been measured in cerebral microvessels of spontaneously and DOCA-salt hypertensive rats, using the specific radioligand ^{125}I-iodohydroxybenzylpindolol (Kobayashi H. et al, J. Neuroch. 34, 1383-1388, 1981). The results show that both in genetic and in experimental hypertension a significant reduction of β-receptor number may be detected, without relevant changes of the affinity values. (Bmax: 154 ± 8 and 110 ± 6 fmol/mg prot., Kd 82 ± 9 and 74 ± 5 pM for Wistar and SHR, respectively; Bmax: 168 ± 9 and 125 ± 4 fmol/mg prot., Kd: 120 ± 7 and 112 ± 9 pM for controls and DOCA-salt treated rats, respectively). The reduction of β-adrenergic receptor number may be the consequence of an altered pattern of central adrenergic transmission or of an increase of plasma catecholamines level. The observed change of β-adrenergic receptors may be involved in the functional alterations induced by hypertension in the brain.

146.5 MAGNESIUM AND GTP AFFECT THE INTERACTIONS OF ANTAGONISTS WITH β-ADRENERGIC RECEPTORS ON MEMBRANES FROM RAT LUNG AND S49 LYMPHOMA CELLS. Margarita L. Contreras*, Barry B. Wolfe, and Perry B. Molinoff, Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, PA.

The effect of Mg^{++} on the binding of antagonists to β-adrenergic receptors was examined. Membranes from rat lung or wild type S49 lymphoma cells were incubated with either ^{125}I-iodohydroxybenzylpindolol (IHYP) or ^{125}I-iodopindolol (IPIN) in the presence of increasing concentrations of Mg^{++} up to 180 mM. In both tissues, concentrations of Mg^{++} above 0.4 mM caused a dose-dependent decrease in the amount of specifically bound IHYP and IPIN. In membranes prepared from wild type cells, IPIN binding was decreased by approximately 40% in the presence of maximally effective concentrations of Mg^{++}. In the absence of Mg^{++}, GTP increased the amount of IHYP bound to membranes prepared from rat lung and S49 lymphoma cells. This effect of GTP on antagonist binding to β-adrenergic receptors has been previously reported in studies of L6 cells (B.B. Wolfe and T.K. Harden, J. Cyclic Nucl. Res. 7:303, 1982). In studies carried out in the presence of GTP, Mg^{++} caused a dose-dependent decrease in the binding of the radioligand. The amount of radioligand bound at each concentration of Mg^{++} was, however, greater than that bound in the absence of GTP. To determine if the effects of Mg^{++} and GTP on binding of antagonists to the β-adrenergic receptor required the guanine nucleotide binding protein (G/F), dose response curves for Mg^{++} were carried out with membranes from cyc^- cells which lack the G/F protein. A Mg^{++} dependent decrease in the amount of IPIN and IHYP bound was observed suggesting that the G/F protein is not required for the effect of Mg^{++} on the binding of antagonists. The GTP induced increase in IPIN and IHYP binding to the β-adrenergic receptor was not seen, however, in studies with cyc^- membranes suggesting that this effect is dependent on the presence of the G/F protein. Scatchard analysis of IHYP and IPIN binding to rat lung and S49 membranes in the presence of 0, 5, and 50 mM Mg^{++} showed that Mg^{++} affected the binding of antagonists by decreasing the affinity of the receptor for antagonists. No effect on the density of receptors was observed. GTP increased the binding of antagonists through a decrease in the K_D value rather than a change in the density of receptors. Scatchard analysis of the binding of IPIN and IHYP to cyc^- membranes revealed an effect of Mg^{++} on the K_D value. The results suggest that the effects of GTP and Mg^{++} on the interactions of antagonists with β-adrenergic receptors are through different mechanisms. (Supported by the USPHS NS 18479)

146.6 ANTIBODIES TO THE β-ADRENERGIC RECEPTOR: FUNCTIONAL CHARACTERIZATION AND RECEPTOR IMMUNOLOCALIZATION IN BRAIN. C.D. Strader*, V.M. Pickel, T.H. Joh, M.W. Strohsacker*, R.G.L. Shorr*, R.J. Lefkowitz*, and M.G. Caron*. Howard Hughes Med. Inst. Lab., Dept. of Med. and Biochem., Duke Univ. Med. Ctr., Durham, NC and Lab. of Neurobiology, Cornell Univ. Med. College, New York, NY.

Antibodies against the frog β-adrenergic receptor (βAR) were raised in rabbits. Adrenergic receptor of the β_2 subtype was extracted from frog erythrocyte membranes in digitonin and purified by affinity chromatography and high performance liquid chromatography (HPLC) according to Shorr, et al. (Proc. Natl. Acad Sci. USA 79: 2778-2982, 1982). Antibodies could be detected in rabbit serum by immunoprecipitation of either purified [^{125}I]-βAR or of solubilized βAR bound to the high-affinity antagonist [^{125}I]-iodocyanopindolol ([^{125}I]-CYP); the titer of antibodies to the βAR was measured as 3.3 pmol βAR bound per ml serum.

Binding of antibody to membrane-bound βAR in frog erythrocyte membranes could be demonstrated by specific labeling with [^{125}I]-goat anti-rabbit IgG. Further characterization of these antibodies showed no interference with antagonist binding to the βAR: incubation of membrane-bound or solubilized βAR with antiserum did not inhibit binding of [^{125}I]-CYP to the receptor and had no significant effect on the K_D of [^{125}I]-CYP binding. In the presence of antibody, purified radioiodinated βAR migrated with an apparent larger molecular size on HPLC, demonstrating the existence of an antibody-receptor complex. The antibody did not bind to βAR solubilized from the turkey erythrocyte (βAR of the β_1 subtype) either labeled with [^{125}I]-CYP or purified and radioiodinated.

Antiserum or purified IgG to the βAR was immunocytochemically localized and compared with localization of tyrosine hydroxylase (TH) in the frog and rat brain. Many regions containing TH labeled processes also contained neurons having specific immunoreactivity for the receptor including, most prominantly, the cerebellum, hippocampus, hypothalamus, neostriatum, and anterior thalamic nuclei. In the hippocampus of the frog, TH was localized by electron microscopy in axons and axon terminals, whereas the immunoreactivity to the βAR was distributed throughout the dendrites and the perikarya. This immunocytochemical localization of the βAR agrees with the receptor distribution reported by Palacios and Kuhar (Science, 208: 1378-1380, 1980) for autoradiography of ligand binding sites using [^{3}H]dihydroalprenolol. Postsynaptic dendritic membranes in these preparations were specifically immunolabeled.

The properties of this antibody promise to make it a useful tool for further characterization and localization of the βAR in its various physiological and pathophysiological states.

146.7 ^{3}H-PARA-AMINO-CLONIDINE BINDING TO α_2-ADRENOCEPTORS IN CAT CORTEX AND SPINAL CORD. J.R. Howe* and T.L. Yaksh* (SPON: R.M. Weinshilboum). Dept. of Pharmacology, Mayo Clinic, Rochester, MN 55905.

Para-amino-clonidine (PAC) has been reported to be a selective ligand for α_2-adrenoceptors in mammalian CNS. We have characterized ^{3}H-PAC binding to homogenates of cat frontal cortex and cat spinal cord. Triplicate incubation tubes on ice received 50 µl of ^{3}H-PAC, 50 µl of various drugs in 0.1% ascorbic acid, and 0.9 ml of tissue suspension diluted 60-fold in 50 mM Tris buffer. For routine experiments, tubes were incubated at 37°C for 10 min and the incubation terminated by rapid filtration over Whatman GF/B filters. The filters were immediately rinsed with three 5-ml washes of ice-cold buffer. Filters were counted by liquid scintillation spectrometry at 35-40% efficiency. Specific binding was defined as that portion of the total that was not displaced by 10 µM (-)-norepinephrine or 1 µM PAC. Specific ^{3}H-PAC binding was saturable and rapidly reversible. Scatchard transformation of data from equilibrium saturation experiments resulted in biphasic plots. K_D and B_{max} values for both the high and low affinity ^{3}H-PAC sites were calculated using computer-assisted nonlinear regression analysis. Mean K_D (nM) and B_{max} (fmoles/mg protein) values obtained from 8 experiments using spinal cord and 4 experiments using cortex are:

	K_{D_H}	B_{max_H}	K_{D_L}	B_{max_L}
Spinal cord	.45	7.1	9.3	56
Cortex	.52	28	9.1	149

Competition experiments were conducted using .13 nM ^{3}H-PAC. K_i values for unlabelled PAC agree well with K_D values for ^{3}H-PAC. K_i values determined for catecholamine stereoisomers indicate that the sites labelled by ^{3}H-PAC exhibit stereoselectivity. The rank order affinity of the stereoisomers for ^{3}H-PAC sites is: (-)-epinephrine > (-)-norepinephrine > (+)-epinephrine > (+)-norepinephrine. Competition experiments with subtype-selective adrenergic agonists and antagonists confirm that the sites labelled by ^{3}H-PAC possess a pharmacologic profile characteristic of α_2-adrenoceptors. We conclude ^{3}H-PAC labels α_2-adrenoceptors in cat frontal cortex and cat spinal cord. (Supported by Mayo Foundation and NS 16541.)

146.8 STRIATAL BETA-ADRENERGIC RECEPTORS REGULATE DOPAMINE RELEASE, T.D. Reisine*, M.F. Chesselet, C. Lubetzki, A. Cheramy and J. Glowinski. Groupe Neurobiology, INSERM U. 114, College de France, Paris, France.

The effect of beta-adrenergic receptor agonists and antagonists on the spontaneous release of ^{3}H-dopamine (^{3}H-DA) continuously formed from ^{3}H-tyrosine was studied both in vitro using rat striatal slices and in vivo in halothane-anaesthetized cats implanted with push-pull cannulae in the caudate nucleus. In vitro, (-) isoproterenol (10^{-6}M) stimulated ^{3}H-DA release whereas (+) isoproterenol (10^{-6}M) did not. The (-) isoproternol effect was blocked by propranolol (10^{-6}M) and practolol (10^{-5}M) but not by phentolamine (10^{-5}M). Furthermore, the stimulation of ^{3}H-DA release was tetrodotoxin (5×10^{-7}M) insensitive. In vivo, (-) isoproterenol (10^{-6}M) facilitated ^{3}H-DA release in the caudate nucleus. Propranolol (10^{-6}M) blocked this action and slightly reduced local ^{3}H-DA release by itself. The short-term effect of (-)-isoproterenol on in vitro ^{3}H-DA release was not accompanied by a change in DA synthesis nor did it appear to be mediated by cyclic-AMP, since cyclic-AMP's actions on ^{3}H-DA efflux and synthesis were blocked by α -methyl-p-tyrosine whereas (-) isoproterenol could still stimulate ^{3}H-DA release under such conditions. Thus, activation of striatal beta-adrenergic receptors, possibly located on DA terminals can potentiate DA release through a cyclic-AMP independent mechanism. These data suggest that noradrenergic neurons or possibly circulating noradrenaline can exert an important control over striatal DA transmission. The implications of such an interaction will be discussed.

146.9 PRESYNAPTIC LOCATION AND AXONAL TRANSPORT OF β_1-ADRENO-RECEPTORS IN RAT BRAIN. B. E. Levin. Lab. of Neuropharmacology, VA Medical Center, East Orange, NJ 07019.

Synthetic enzymes and other proteins are generally thought to be synthesized in the neuronal cell body and anterogradely transported to the nerve terminal. Hypothalamic lesions in the ascending noradrenergic axons of locus coeruleus neurons with 6-hydroxydopamine have been shown to produce accumulation of norepinephrine synthetic enzymes proximal to the lesion. In the present study, similar injections of 2 µl 6-hydroxydopamine (4 µg/µl) into the median forebrain bundle in the left rostral hypothalamus led to the progressive accumulation of {^{3}H} dihydroalprenalol ({^{3}H} DHA) binding over a 2 d period proximal to the lesion. Binding activity plateaued from 2-5 d and accumulation could be completely blocked by a second injection in these axons closer to the cell body. Scatchard analysis showed that the accumulation proximal to the lesion after 2 d was due to an increased number of binding sites (B_{max}: lesioned = 173 ± 10, versus unlesioned = 128 ± 11 fmol/mg protein; $p < 0.05$) rather than altered affinity which was actually lower on the lesioned (11.5 ± 0.9 nM) compared to the unlesioned side (8.7 ± 1.0 nM; $p < 0.05$). Competitive binding studies with the β_2-adrenoreceptor antagonist, Zinterol, showed that 56.8 ± 4.6% of hypothalamic membrane receptors were of the β_2- and 43.2 ± 2.5% were of the β_1-sub-type in unlesioned brain. Accumulation of {^{3}H} DHA binding proximal to the lesion was exclusively due to an increase in the number of β_1-receptors (105 ± 15 lesioned versus 55 + 8 fmol/mg protein unlesioned; $p < 0.005$) since the number of β_2 receptors was unchanged. There was no comparable transport of α_1 ({^{3}H} WB-4101) binding sites in this system. Therefore, β_1-adrenoreceptors appeared to be localized to, and undergo transport in, presynaptic noradrenergic neurons in rat brain.

147.1 SYNAPTIC INTERACTIONS IN THE OLFACTORY LOBE OF THE MOTH *MANDUCA SEXTA*. I. D. Harrow and J. G. Hildebrand. Dept. of Biological Sciences, Columbia University, New York, N. Y. 10027.

Olfactory signals detected by primary sensory neurons in the insect antenna are conveyed by afferent axons in the antennal nerve to the antennal lobe (AL) of the brain for synaptic processing. The AL contains two general classes of interneurons: local and output. Matsumoto and Hildebrand (*Proc. Roy. Soc. Lond. B215:* 249-277, 1981) described the morphology and responses of these neurons to various odors applied to the antenna. The work presented here is aimed toward gaining an understanding of information processing in the olfactory pathway of the moth *Manduca*. Our approach is to probe the synaptic interactions between the different types of neurons in the AL.

Intracellular recording from, and staining of, individual neurons in the AL are accomplished with glass microelectrodes filled with 5% Lucifer Yellow CH. In response to electrical stimulation (0.1 msec) of the ipsilateral antennal nerve, AL type-I output neurons show complex postsynaptic responses typically comprising a relatively early, compound ipsp (graded with different stimulus intensities) followed by a compound epsp and burst of spikes. The compound ipsp (up to 10 mV) can follow afferent stimuli only when they are delivered at low frequency (< 10 Hz), and the latency of the ipsp, measured from the stimulus artifact, is relatively long (∿ 10 msec). These observations suggest that the inhibitory input is probably indirect via at least one inhibitory interneuron. Injection of hyperpolarizing current simultaneously with afferent stimulation reverses the sign of the psp, indicating that the ipsp is due to chemical neurotransmission. Moreover, the reversal potential is negative with respect to the resting potential, suggesting that an increase in Cl^- or K^+ conductance underlies the ipsp. Instantaneous frequency analysis of the spiking activity in the same output neuron reveals that the late excitatory component of the complex response (∿ 150 Hz peak instantaneous frequency) is time-locked to the electrically evoked afferent input. This suggests that a likely function of the early inhibitory input to an output neuron is to synchronize the late excitatory responses. This would enhance the signal-to-noise ratio at this level in the olfactory pathway.

In future experiments we shall seek to identify the antennal afferent inputs that evoke the complex response by applying odors to the antenna. We also plan to characterize the neurons that mediate the inhibitory and excitatory inputs to the output neurons of the AL.

These studies have been supported by NIH grant AI-17711 and NSF grant BNS 80-13511.

147.2 THE DYNAMIC SENSITIVITY OF A LOCUST MULTIPOLAR JOINT RECEPTOR. Andrew S. French and Janice E. Kuster*. Department of Physiology, University of Alberta, Edmonton, Canada. T6G 2H7

Insects contain two morphologically distinct types of mechanoreceptors. Type I, or cuticular mechanoreceptors, are bipolar neurons with a single ciliary dendrite terminating in a dense tubular body, surrounded by a dense dendritic sheath. They are always associated with the cuticle. Type II, or multipolar sensilla, have many dendrites which terminate in unspecialized naked endings. They are found in deeper tissues, lining the internal organs, associated with muscles and in the joints. Studies of sensory transduction in insect mechanoreceptors have concentrated on the Type I sensilla because their location makes them relatively easy to stimulate and to record from. A few studies have been made of the Type II receptors in leg joints, which can be stimulated by moving the limbs.

Type I sensilla are often rapidly adapting and in the frequency domain their response increases with frequency while there is a constant phase lead of the response relative to the stimulus. This type of behaviour is characteristic of a system which performs fractional differentiation of the input signal. Although a number of suggestions have been made, there is no satisfactory explanation for the location or the mechanisms of such fractional differentiation. We have suggested that the visco-elastic properties of the tubular body may contribute to the dynamic behaviour, in analogy to similar contributions by mechanical components in vertebrate mechanoreceptors such as the Pacinian corpuscle or the muscle spindle.

In the present work we have performed linear systems analysis of one of the multipolar joint receptors in the metathoracic leg of the locust, using both sinusoidal and random mechanical stimuli to the joint while recording the afferent action potentials in the axon extracellularly. The sensillum is tonically active with a mean rate of firing which increases in proportion to joint extension. Sinusoidal stimulation produces modulation of the rate of firing around this mean rate to give a smoothly sinusoidal response when measured as the peri-stimulus time histogram. However, phase-locking of the action potentials to a repetitive stimulus causes distortion of the frequency response function above about 1 Hz. Random stimulation substantially reduces the phase-locking problem and allows the frequency response function to be determined up to about 10 Hz. The results, measured under a range of different stimulus conditions, indicate that a fractional differentiator model can account for the behaviour of these Type II sensilla, suggesting that adaptation behaviour arises in the dendritic membrane rather than in the mechanical components surrounding insect mechanosensory cells.

147.3 SENSITIVITY TO DIMMING AS A FUNCTION OF BACKGROUND LIGHT INTENSITY IN THE BARNACLE'S VISUAL SYSTEM. Kathleen A. French and Ann E. Stuart. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27514.

The slight dimming of light evokes a protective reflex response in barnacles. In the giant barnacle, it has been shown that dimming leads to a hyperpolarization of the photoreceptors (PRs) and a depolarization of second- and third-order cells (Stuart and Oertel, 1978). Very small changes in PR voltage can lead to impulses in third-order cells (Shaw, 1972). To begin to explore the basis of this sensitivity, we have studied the responses of median PRs and third-order cells (A-cells) to step decreases in light. Backgrounds ranged over 6 log units ($10^{-7.2}$ to 0.33 mW/cm^2).

By recording extracellularly from the A-cell's axon, we determined the minimum percent decrease in illumination ($\Delta I/I$) required to evoke impulses from it at different background intensities (I). For a dim background ($10^{-5.4}$ mW/cm^2), $\Delta I/I$ was as high as 0.9, while for a bright background of 10^{-1} mW/cm^2 the threshold $\Delta I/I$ dropped to 0.03. Thus, these cells are increasingly sensitive to dimming as the background becomes brighter.

To obtain a more accurate measure of the A-cell's response, we recorded intracellularly from its soma. The preparation was perfused with saline containing tetrodotoxin to block the cell's impulses, so that the amplitude of the excitatory postsynaptic potential (EPSP) elicited by dimming could be measured. At any given background, the amplitude of this EPSP increased rapidly with $\Delta I/I$ over a range that narrowed with increasing background intensity. The dimming of brighter backgrounds by any given value of $\Delta I/I$ produced larger EPSPs. For example, in one experiment, decreasing the light intensity by half produced an 8mV EPSP at $10^{-4.2}$ mW/cm^2 and a 16 mV response at $10^{-2.4}$ mW/cm^2.

To determine whether changes in PR voltage could account for the effect that background has on the A-cell's sensitivity, we recorded intracellularly from PRs near their terminals. In contrast to the observations on A-cells, the voltage changes in a PR remained nearly constant when different backgrounds were dimmed by the same values of $\Delta I/I$ below about 0.8. Above that value the responses diverged from one another.

We conclude that, over a range of 4-5 log units of light intensity, A-cells are increasingly sensitive to the dimming of a constant light as this background is made brighter. This increase in sensitivity cannot be due simply to the size of the voltage change in the receptors' terminals.

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Shaw, S. J. Physiol. (London), 220:145-175, 1972.
Stuart, A. and D. Oertel. Nature, 275:287-290, 1978.

147.4 CONTOUR SENSITIVITY AND ELECTROANATOMY OF CRAYFISH SUSTAINING FIBERS. B. Waldrop, M.D. Kirk and R.M. Glantz . Dept. of Biology, Rice University, Houston, TX 77251

Crayfish sustaining fibers (SFs) are tonic light-ON interneurons identified individually on the basis of their corneal receptive fields. Intracellular recordings show large (20-40 mV) EPSPs which can be affected by extrinsic current. I-V and I-f relationships are quite linear, allowing comparison of voltage and frequency data. Dye injections have revealed that the SFs have uniplanar dendritic arbors in a common layer of the second optic ganglion, the Medulla. The location of each cell's dendrites within the retinotopic columnar organization of the medullary neuropil predicts that cell's corneal receptive field.

Sensitivity of a SF to light varies within its receptive field. Detailed contour sensitivity maps have shown that a correlation exists between areas of high sensitivity on the cornea and the density of secondary and tertiary dendritic branches in the corresponding region of the neuropil, but apparently not to a degree which is sufficient to explain the observed variation of three log units of light sensitivity.

A compartmental electrtonic model of the SFs was constructed to determine if dendritic location and electrotonic decrement could play a role in contour sensitivity. The general solutions of the cable equations for DC current spread through dendritic trees (Rall, W., Exp.Neurol., 1:491-527, 1959) were used, along with an estimate of the specific axoplasmic resistivity (55 Ω-cm) and the geometry of the dye-filled SFs, to iteratively calculate a value for Rm, the specific membrane resistivity, which best predicts the observed input resistance (mean Rin for 14 preparations, 7.8 MΩ). The range of values for different cells is 8000-12,000 Ω-cm^2. We then applied Rall's algorithms to determine the decrement of DC potentials from various points on the dendritic tree to the probable site of spike initiation in the axon. A large transverse process seen in each SF, which serves as a summing point for the dendritic branches, appears to minimize differences between distal and proximal branches. Our computations indicate that for equal currents injected into the most proximal and the most distal terminal branches, the voltages at the spike initiating zone should differ by about 10%. Therefore the observed differences in sensitivity must be due to other factors, such as synaptic density or magnitude of synaptic conductance changes. The linear I-V, I-f, and spatial summing properties of the SFs indicate that their discharge is a remarkably faithful reflection of their synaptic inputs.

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147.5 **LATERAL INHIBITION MECHANISMS OF MOVEMENT DETECTORS.** **R.B. Pinter Departments of Electrical Engineering and Zoology, University of Washington, Seattle, Washington, 98195.**

Many cells of visual systems respond preferentially to small objects which are moved or dimmed or brightened. In some cases, (e.g., dragonfly ventral cord object-movement units) the unit response resembles a spatial logic operator of an "exclusive-or" form (Olberg, R. J. Comp. Physiol. 141, p. 327, 1981) but in others the preference or selectivity functions are less steep (Palka, J. J. Insect Physiol. 13, p. 235, 1967, in locust DCMD).

Lateral inhibition of the Hartline-Ratliff type accounts well for response on peripheral levels in Limulus lateral eye, where the inhibition is linear up to large variations in the input variables even though Lange corrections (Barlow, R.B., Jr., and Lange, G.D. J. Gen. Physiol. 63, p. 579, 1974) are indicated for large amplitudes of eccentric cell generator potential.

A system of linear lateral inhibition does not however account well for movement detectors' sharp small object selectivity which is inherently nonlinear. Given a simplified form of Hartline-Ratliff linear lateral inhibition, in one dimension (i), where the ith cell's transmembrane potential is represented by e_i, the input is I_i and k a constant,

$$\frac{de_i}{dt} = I_i - e_i - ke_{i-1} - ke_{i+1}, i = 1,2,\ldots \quad (1),$$

it can be shown that the second order unperturbed form of non-linear lateral inhibition

$$\frac{de_i}{dt} = I_i - e_i - k_1 e_i e_{i-1} - k_1 e_i e_{i+1}, i = 1,2,\ldots \quad (2)$$

has the property of sharper small object selectivity than (1), along with movement detector properties not found in (1): input intensity dependent selectivity and latency. The latter two properties will not be found in any linear system. If the terms $-(k_2 e_i e_{i-2} + k_2 e_i e_{i+2})$ are added to equations (2) object edge sensitivity is considerably reduced.

In the compound eye, the inputs I_i are derived from visual space by approximately Gaussian angular sensitivity functions. Including this smooths the object angular sensitivity functions but causes the preferred size to vary with input intensity and the latency to vary with size of object, as found in movement detectors.

It is of interest that one kind of lateral inhibition produces several nonlinear properties of movement detectors. These properties are inter-related and not a one-to-one mapping on any coefficients or terms of the inhibition.

147.6 ACTIVATION OF VIBRATION RECEPTORS INITIATES SWIMMING IN A SEMI-INTACT LEECH PREPARATION. P. D. Brodfuehrer and W. O. Friesen. Dept. Biol., Univ. of Virginia, Charlottesville, VA 22901.

Leeches, Hirudo medicinalis, respond to surface waves by initiating swimming movements toward the source of the waves (Young, Dedwylder and Friesen, J. Comp. Physiol., 144:111, 1981). Detection of surface waves is mediated by vibration receptors located at the sensilla (Friesen, J. exp. Biol., 92:255, 1981). Fourteen sensilla are located circumferentially over the middle annulus of each mid-body segment, while fewer sensilla are located on the head and tail segments. Previous attempts to initiate swimming in a semi-intact leech preparation by wave stimulation have been unsuccessful. Using the fact that serotonin increases the probability of swimming in an isolated nerve cord (Willard, J. Neurosci., 1:936, 1981), we applied 50 µM serotonin to a semi-intact preparation and observed an increase in the number of spontaneous swimming episodes. When the rate of spontaneous swimming episodes was about two per 10 minutes, we could elicit up to 20 swimming episodes by wave stimulation in a 10 minute period. (In an active preparation swimming could be reliably initiated by surface waves 75% of the time.) Once wave stimulation ceased, the rate of spontaneous swimming episodes again returned to one or two per 10 minutes. This indicates that semi-intact leeches were swimming in response to wave stimulation and that this preparation is an accessable system in which to study the mechanism by which vibration receptors activate the swimming rhythm.

The precise locus of serotonin action is not yet known. We have characterized the afferent response of the vibration receptors to near-field stimulation both with and without serotonin and have observed no difference in the amplitude (250 µV) or duration (10-12 ms) of the vibration response in the dorsal posterior nerve. Serotonin does, however, increase the activity in the connectives and in the dorsal posterior nerve. For example, the dorsal excitor motor neuron, cell 3, occasionally fires 1-5 spikes in response to surface waves in the absence of serotonin. When the semi-intact leech is bathed in serotonin, surface waves evoke bursts of spikes in cell 3 which are reminiscent of the type of activity seen in cell 3 during swimming. Hence, serotonin appears to be exerting its effect centrally. Supported by NIH grant NS 14965.

147.7 INTRACELLULAR STIMULATION OF MECHANOSENSORY CELLS INITIATES SWIMMING IN THE MEDICINAL LEECH. E. A. Debski and W. O. Friesen. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

Tactile stimulation of an innervated body wall flap elicits swimming episodes in an otherwise isolated chain of leech ganglia . Any or all of the classical mechanoreceptors of the leech, the touch (T), pressure (P), and nociceptive (N) cells, could be mediating this swimming response. Yet published observations have suggested that swimming cannot be reliably obtained by individual stimulation of T, P, or N cells (Weeks and Kristan, J. exp. Biol. 77: 72, 1978). We have found that, contrary to these previous findings, intracellular stimulation of all three types of mechanoreceptors can elicit swimming episodes. Intracellular stimulation of individual N cells is most effective for swim initiation, P cell stimulation is somewhat less effective, and intracellular stimulation of a single T cell rarely leads to swimming activity. However, simultaneous stimulation of two T cells in adjacent ganglia reliably evokes swimming. The effectiveness of the three cell types fatigues rapidly; this observation could account for the failure of previous researchers to recognize the swim initiating capabilities of these cells. Stimulation of N cells, for example, will elicit only about four swim episodes in succession. For P and T cells, fatigue occurs even more rapidly. We found that the ability to initiate swimming can be temporarily restored to N cells by electrical stimulation of a segmental nerve (DP), while T cell effectiveness recovers following mechanical stimulation of a body wall flap attached to the ventral nerve cord.

Our attempts to elicit swimming activity in a nearly isolated nerve cord by lightly stroking a body wall flap revealed that such stimulation is initially very effective. However, this effectiveness also decreases with repeated stimulation, so that following five or six stimulus presentations, swimming activity is no longer produced. The ability of body wall stroke to initiate swimming recovers spontaneously or following the mechanical manipulation of another body flap. The T cell that innervates the body flap fires in rapid bursts while the remaining T cells in the ganglion are usually silent (N and P cells fire rarely, if at all, during body wall stroke). The effect of this stimulus on T cells in adjacent ganglia has not yet been examined. Experiments are currently underway to determine if T cell activity is necessary for this type of swim initiation.

Supported by NIH grant NS 14965.

147.8 NEURAL BASIS OF ULTRASOUND AVOIDANCE IN THE CRICKET TELEOGRYLLUS OCEANICUS. T.G. Nolen and R.R. Hoy, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Tethered, flying crickets respond to pulses of ultrasound by bending their abdomen, legs and antennae away from the sound source (Moiseff, Pollack and Hoy, P.N.A.S. 75:4052). In the field, this behavior would allow them to fly away from echolocating, insectivorous bats, possible predators of crickets. We find that the ultrasound response is different from that elicited by the species calling song (i.e. 4.5 to 5 kHz). We show that an identified auditory interneuron (Interneuron 1, Int-1) is an important component of the neural circuit mediating ultrasound avoidance.

Tethered, flying crickets respond to single, 30 msec ultrasound pulses (30 kHz) by making short latency (35 msec) phasic abdomenal flexions away from the side of stimulation at a threshold of 57.7 ± 5.7 dB SPL (mean ± SD, N = 65 animals). However, single pulses near the calling song frequency (e.g. 5 kHz) elicit no response in 85% of those tested, even at 100 dB (a negative response was elicited in 15%, but only at >=100 dB, N = 56 animals). As a comparison, the threshold to the temporally patterned calling song is 55 dB.

Intracellular recordings show that Int-1 is broadly tuned between 18 and 50 kHz (thresholds between 40 and 60 dB) but has a high threshold peak of sensitivity near the calling song (threshold at 5 kHz of 50 to 70 dB). 5 kHz mediates both inhibition (IPSP's with reversal potentials near resting potential) as well as excitation, such that spike rates at threshold +10 dB are lower than those elicited by ultrasound. As a result, Int-1 is maximally excited by ultrasound (between 20 and 40 kHz).

Finally, Int-1 appears to play a direct role in the behavioral response to ultrasound. When a 30 kHz pulse was presented to a flying cricket, a burst of action potentials was elicited in Int-1 (spike rate approx. 200 Hz), followed by activation of the contralateral abdominal steering muscles (the dorsal longitudinal muscles or DLM's). When the spike rate in Int-1 was reduced below a critical level by hyperpolarizing current injection, the DLM response was abolished. Above the critical level, the DLM response was positively correlated with the spike rate in Int-1. The reduction in the DLM response was reversible.

In the absence of ultrasound stimulation, electrical excitation of Int-1 in a flying animal was followed by strong activation of the contralateral DLM's. The DLM's could not be activated unless the animal was flying.

These results strongly suggest that the bilateral pair of Int-1's are necessary and sufficient components of the ultrasound avoidance behavior.

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147.9 ELECTRONMICROSCOPY RESOLVES SPECIAL JUNCTIONS IN "TRANSSYNAPTIC" COBALT-COUPLED NERVE CELLS IN GIANT FIBRE SYSTEMS (GFS) OF FLIES (Drosophila, Musca & Calliphora). U.Bassemir*, N.J.Strausfeld, J.P.Bacon* and R.N.Singh*. EMBL, D-69 Heidelberg, West Germany; SUNY Albany, Albany, New York 1222; T.I.F.R., Bombay, India.

Stereotyped passage of cobalt ions occurs between certain neurons of the GFS in Drosophila, Musca and Calliphora. In Drosophila paired homolateral Giant Descending Neurons (GDNs) are cobalt-coupled to antennal inputs, small field visual interneurons (Col As), Tergotrochantal Motorneurons (TTMs) and Peripherally Synapsing Interneurons (PSIs). GDNs are also coupled in the brain via several Giant Commissural Interneurons (GCIs) and are directly coupled at their terminals in the mesothoracic ganglion. In Musca and Calliphora, GDNs are not coupled to PSIs or to GCIs but are coupled to certain male-specific visual neurons in male flies (MLG 1).

X-ray emission spectroscopy of fine grain electron-dense material in filled cells of Musca generated by a highly sensitive silver intensification method for use on intact brains shows the precipitates to be cobalt (sulphide)-silver deposites. CoS-Ag precipitates are localized at mitochondria, neurofilaments and pre- and postsynaptic membrane specializations. In cobalt-coupled neurons the secondarily filled cells contain fewer CoS-Ag grains which are first resolved at synaptic areas and in mitochondria. CoS-Ag precipitates are also localized at special gap junction-like structures that are shared by the pre- and postsynaptic membranes of cobalt-coupled neurons (e.g Col A cells and GDNs, in Musca). Cells presynaptic onto GDNs, but never cobalt-coupled do not exhibit these structures and do not contain CoS-Ag.

Iontophoretic injection of the fluorescent dye, Lucifer Yellow, into GDNs of Musca after electrophysiological recordings can also reveal Lucifer-coupling between GDNs at their endings, between GDNs and Col A cells and,in one case, between a GDN and the MLG 1 neuron in a male. Dye coupling and cobalt coupling of GDNs to other neurons in Musca is equivalent although structural resolution with cobalt-silver is superior.

We suggest that "transsynaptic" cobalt-coupling occurs specifically between neurons sharing gap junction-like structures and that cobalt-coupled neuronal pathways are possibly functional pathways in the insect central nervous system. We are pursuing the functional nature of cobaltomissive pathways by intracellular recordings and by electronmicroscopy of the GDN to TTM and PSI connections in Drosophila. Cobalt-coupling is also being used by us for analysing abnormal neuronal pathways in mutants of Drosophila affecting the sensory input and for the analysis of cell arrangements in the mouse cerebellum.

147.10 HIGH VOLTAGE ELECTRON MICROSCOPE STUDY OF RETINAL DEGENERATION IN DROSOPHILA, William S. Stark, Kiran Srivastava* (Div. Biol. Sci., Univ. Missouri, Columbia, MO 65211) and Stanley D. Carlson* (Dept. Entomology, Univ. Wisconsin, Madison, WI 53706).

We used High Voltage Electron Microscopy (HVEM) to study the defects involved in retinal degeneration of the compound eye and underlying neuropile in white-eyed Drosophila. This technique allows the convenient use of relatively thick (0.25-0.5 μm) sections with good resolution of cells, organelles and membranes. We studied 3 types of retinal degeneration: 1) induced by intense light (blue and UV); 2) caused by mutant alleles of the rdgA gene (BS12 and PC47); and 3) caused by the mutation $rdgB^{KS222}$.

1) Bright blue and UV light damages the eye (Miller et al. 1982, Invest. Ophth. Vis. Sci. Suppl. 22, p.66). Cytoplasm of the retinular cells is not homogeneous but appears to be coagulated into a series of coarse electron dense strands that extend parallel to the microvillar direction. The microvilli are reduced and appear nonpatent. Large fluid filled spaces are proximal to the basement membrane. Second order cells are present but disorganized by gliosis. There are no remains of retinular cell axons. The threshold for damage is between 18.5 to 18.7 log quanta/$cm^2 \cdot s$ (for blue) and below 17.9 (for UV) given 30 s stimuli.

2) The mutants $rdgA^{BS12}$ and $rdgA^{PC47}$ cause severe vs gradual degeneration (Johnson et al. J. Insect Physiol. 28, 1982, 233-242). PC47 has a normal retina and visual projection when newly-emerged. The R1-6 photoreceptor subset (somata and axons) of PC47 aged 1 wk is in an advanced state of lysis. R7/8 are better preserved. Higher order neurons look normal. In newly-emerged BS12, R1-6 have small rhabdomeres and few mitochondria. R7 and R8 are recognizable but their rhabdomeric microvilli are deranged, divided into double sets or in several orientations. The presence of pseudocartridges beneath the basement membrane shows that an axonal projection begins. But where the synaptic cartridges should be in the lamina ganglionaris (first optic neuropile) there are no axons, only a gliosis. Second order neurons, if present, are disorganized. The retina in week-aged BS12 is very degenerate, though the lamina looks curiously less degenererate than in PC47 due to lack of degenerating axon terminals.

3) These results can be compared with recent studies of white-eyed $rdgB^{KS222}$ (Stark and Carlson, Cell and Tiss. Res. 1982 in press) whose degeneration is light-induced. After 1 wk of cyclic lighting, R1-6 show dense, nonpatent microvilli and cytoplasm that seems coagulated into an electron-dense reticulum. R1-6 axons are degenerate and in the process of phagocytosis by adjacent epithelial glia. R7/8 and higher order cells are normal.

In summary, Drosophila have different retinal degenerations.

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147.11 CERCAL SENSORY SYSTEM IN A PRIMITIVE INSECT. John S. Edwards. Max Planck Institut fur Verhaltensphysiologie, Seewiesen, Federal Republic of Germany and Zoology Department, University of Washington, Seattle, WA., 98195.

The cercal sensory system now known from a series of pterygote insects, notably orthopteroids, has its presumed origin among the primitively wingless insects (Apterygota). The Thysanura (silverfish, firebrats) are modern apterygotes that may be considered to reflect the level of cercal sensory organization of the stem pterygotes.

In the thysanuran Thermobia domestica the median caudal filament and the paired lateral cerci all bear mechanoreceptor hairs of at least three types arranged in a repeating pattern of segments on which distal arrays of filaform sensilla are similar to the Notoptera. The afferent nerve of the median filament is devided laterally, and is fused with the lateral cercalnerves. Central projections of median and lateral nerves partially overlap in three regions of arborization.

Within the CNS four giant interneurons occupy positions that appear to be homologous with those of orthopteroid counterparts. Extensive presynaptic sites occur on at least 2 giant interneurons in the penultimate ganglion. Type 1 synapses occupy sites on circular axon profiles of cells ca.25μm in diam. Post synaptic cells are generally small (ca.0.15μm in diam,) grouped in parallel sets of up to 12. with triads frequent. These synapses are the first reported to occur directly on giant axons of an orthopteroid insect.

Behavioral responses indicate an ability to localize sources of mechanoreceptor stimuli delivered as directional airpuffs from the posterior sector. The behavior of animals deprived is not significantly different from normal. The caudal filament alone can mediate escape responses to airpuffs. The functional relationship of input to the terminal ganglion from lateral cerci and caudal filament remains obscure.

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148.1 CYTOCHEMICAL LOCALIZATION OF TYPE I HYDROGEN IN CIRCUMVENTRICULAR ORGANS AND THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM. J.Y. Summy-Long, R. Salisbury*, M. Marietta* and J. Weisz*. Dept. of Pharmacol. (J.S.-L), Div. Rep. Biol., Dept. Ob/Gyn, M.S. Hershey Medical Ctr., Penn State Univ., Hershey, PA 17033.

Using cytochemistry, it is possible to differentiate between two types of reducing equivalents, termed Type I and Type II hydrogen (H), made available from NADPH. The NADPH is generated in cytoplasm by dehydrogenases of the hexose-monophosphate shunt (e.g. glucose-6-phosphate dehydrogenase, G6PD). In peripheral tissues, Type I H is of functional importance in hydroxylation of steroids and drugs. Cellular utilization of Type I H in these tissues requires its transfer from NADPH along the electron-transport chain to the terminal oxidase, cytochrome P-450. Activities of G6PD and the NADP hydrogen-transport chain can be identified histochemically using neotetrazolium chloride. In this system,Type I H generated by the oxidation of glucose is trapped by neotetrazolium as it passes along the cytochrome chain. As neotetrazolium is reduced,it form a blue formazan precipitate. Adult male Sprague Dawley rats (300-400g) were decapitated and different portions of the brain frozen (-70°C) in n-hexane. Saggital sections of brain were cut at 14μ in a cryostat. Sections were incubated in medium containing 5mM glucose-6-phosphate,3mM NADP, 10mM KCN and 4.5mM neotetrazolium chloride dissolved in 0.05M glycyl glycine buffer, pH 8.0, which contained 20% polyvinyl alcohol to prevent loss of soluble enzyme. The medium at 37°C was saturated with nitrogen just before use. After incubation for 20 min at 37°C,sections were rinsed, then fixed and counter-stained with methyl green-pyronin. A regional localization of Type I H was observed. Formazan was localized in cells of circumventricular organs including the subfornical organ,the vascular organ of the lamina terminalis,subcommissural organ and pineal gland. Dense reaction product was found in large neurons in the subfornical organ and in the apical cytoplasm of columnar cells in the subcommissural organ. Type I H was prominent throughout the hypothalamo-neurohypophysial system. Formazan precipitate was seen in some,but not all,magnocellular cells in the supraoptic and paraventricular nuclei,the zona interna of the median eminence and the neurohypophysis. When present in cells,the formazan was extra-nuclear but frequently clustered in peri-nuclear zones. Formazan precipitate was not associated with capillary endothelial cells,but was prominent in arterioles within the CNS. Identification of factors that modulate Type I H formation at these sites is possible by microspectrophotometry. This approach will be useful in delineating the functional significance of the presence of Type I H in these, as well as other,sites in the brain. Supported by Grants #HL 25726 and NICHHD #HD 09524.

148.2 ESTROGEN ALTERS NERVE CELL NUCLEOLI IN RAT HYPOTHALAMUS. R.S. Cohen* and D.W. Pfaff. Dept. of Anatomy, Univ. of Illinois at the Med. Center, Chicago, IL. 60612 and The Rockefeller University, New York, N.Y. 10021.

Estrogen is accumulated from the blood by nerve cells in the ventromedial nucleus (VMN) of the hypothalamus and can facilitate female reproductive behavior by acting on this region of the brain. In hypothalamus and pre-optic area, such estrogen effects apparently involve RNA and protein synthesis. Through their connections to the midbrain, neurons in the ventromedial hypothalamus influence the circuitry for female reproductive behavior, and this appears to comprise a mechanism for sexual motivation. If estrogen effects on behavior include alterations of nerve cell RNA synthesis, estrogen treatment adequate to induce feminine behavior should alter the appearance of ventromedial hypothalamic nerve cell nucleoli, primary sites of RNA synthesis in these as in other cells. We, therefore, ovariectomized 12 female rats and waited one month for estrogen levels to decline. Then, six were given daily injections of 10μg estradiol benzoate, while six control animals were given vehicle injection only. After 15 days of injection, all estrogen-treated rats showed lordosis behavior, and none of the controls did. All rats were perfused and brain tissue dissected and processed according to standard electron microscopic procedures. Thick and thin sections through the ventrolateral portion of the VMN of the hypothalamus, which contains a high density of estrogen-binding cells, were taken for light and electron microscopy. With light microscopy we noticed protuberances on the surface of some neuronal nucleoli, which were more frequent in the ventromedial hypothalami of estrogen-treated than in control female rats. Cells with protuberances on the nucleolar surface occurred more than twice as often in the estrogen-treated compared to the control group (58% of ventromedial neurons in estrogen-treated rats had nucleolar protuberances, compared to 27% in controls). Ultrastructural examination of the VMN of the hypothalamus revealed cells with aggregations of electron dense material corresponding to the nucleolar surface features we described above. At high magnification, this material can be seen to be separated from the nucleolus by a narrow gap, penetrated by strands of this electron dense material which connect it to the main portion of the nucleolus. Steroid hormone actions in many tissues require an increased capacity for protein synthesis and so involve a greater rate of synthesis of ribosomal RNA. Previous ultrastructual results (Cell Tissue Res. 217:451, 1981) indicated increased synthesis of a secretory product by the large amounts of rough endoplasmic reticulum in estrogen-activated hypothalamic cells. This would require increased synthetic rates for rRNA in these nerve cells, which would be reflected at the nucleolar level. Supported by NIH grants NS 15889 and HD-05751.

148.3 IMMUNOREACTIVITY TO NEUROPHYSINS I AND II IN DYE-COUPLED MAGNOCELLULAR HYPOTHALAMIC NEURONS. P. Cobbett*, K. G. Smithson*, B. A. MacVicar & G. I. Hatton. Neuroscience Program and Psychology Dept., Michigan State University, E. Lansing, MI, 48824.

Oxytocin (OT) and vasopressin (VP) are released from the neurohypophysial terminals of hypothalamic neurons. To achieve sufficient plasma hormone concentrations, homotypic neurons must either fire in synchrony or have a high degree of coordinated excitability. Such activity may be produced or facilitated by electrotonic coupling of neurons, which is indicated where dye-coupling of neurons is present. We have investigated the presence of OT and VP in neurons injected with dye in hypothalamic slices where dye-coupling has previously been shown (Science, 211:1187, 1981). Slices were prepared from 30-70 day-old rats of both sexes. The slices were incubated in gassed (95% O_2, 5% CO_2) medium (310 mosmol). Neurons of the paraventricular nucleus (PVN) were injected intracellularly with Lucifer Yellow CH. Precautions to prevent artifactual dye-filling of neurons were taken. After injection, slices were fixed in paraformaldehyde, dehydrated and cleared in methyl salicylate. Tissue was then briefly viewed under epifluorescence and the type and number of neurons containing dye were determined. Slices were then rehydrated, immersed in Bouin's fixative, dehydrated, embedded in polyethylene glycol, and sectioned at 5 μm. Mounted sections were alternately reacted with antibodies to the neurophysin (NP) associated with OT (NPI) or VP (NPII). Subsequent incubations with a biotinylated secondary antibody, avidin-biotin-HRP complex, and 3,3'-diaminobenzidine produced a brown reaction product. Sections were examined to determine the immunocytochemical identity of dye-filled neurons. To date, 82 PVN injections have been made and all dye-filled neurons were magnocellular. Sixty-eight single cells and 14 dye-coupled pairs were observed: 2 pairs were dendro-dendritically coupled, and the others were apparently coupled dendro- or soma-somatically. Of the single neurons, 10 have been identified immunocytochemically: 4 were NPI positive and 6 were NPII positive. Three soma-somatically coupled pairs were NPII positive, but no dendro-dendritically coupled pairs have yet been identified. These data are evidence that dye-coupling in the PVN is genuine. If artifactual, then "coupled" neurons would contain different hormones in some instances since OT and VP neurons coexist in close proximity in the nucleus. The hypothesis that coordinated activity of homotypic neurons may be produced by electrotonic coupling is also supported by the data. Taken together these data suggest that VP neurons are coupled soma-somatically, but that OT neurons, if coupled, may have dendro-dendritic junctions.

This research supported by NIH Grants NS 09140 and NS 16942.

148.4 SUPRAOPTIC NEURONS IN RAT HYPOTHALAMO-NEUROHYPOPHYSIAL EXPLANTS: ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL CHARACTERIZATION OF ELECTROPHYSIOLOGICALLY-IDENTIFIED MAGNOCELLULAR NEURONS. T.A. Reaves, Jr., M.T. Libber* and J.N. Hayward. H. Houston Merritt Electron Microscopy Lab., Dept. Neurology and Neurobiology Program, University of North Carolina, Chapel Hill, N.C. 27514.

Using a double-labeling technique which combines immunocytochemistry with intracellular-recording and -injection of Lucifer Yellow (LY), we described three chemical cell types within the magnocellular (MgC) neuroendocrine system (JCN 193:777'80). We now report the combination of this double-labeling technique with a new procedure that produces insoluble osmophilic polymers in LY injected cells (Maranto, Soc.Neurosci.Abstr. 7:418'81). We find that electrophysiologically studied rat supraoptic (NSO) MgC neurons can be LY-injected, immunocytochemically identified, UV-irradiated and studied by light(LM)- and electron(EM)-microscopy.

Using rat hypothalamo-neurohypophysial explants (Sladek and Knigge, Endocrin. 101:1834'77), we recorded intracellularly from pituitary-antidromically identified NSO neurons with 2% LY-filled glass micropipettes and iontophoretically-injected (5-10 nA,DC) those neurons with LY. Fixed explants were vibratome sectioned (30-50 uM). LY-filled neurons were located with fluorescence microscopy and chemically identified using primary rabbit anti-rat neurophysin antiserum (RN#4, Dr. A.G. Robinson) and a secondary antiserum tagged with rhodamine. Sections containing double-labeled neurons were soaked in a diaminobenzidine (DAB) solution and irradiated under UV light in DAB until LY fluorescence faded below visibility. Irradiated sections were then osmicated, embedded in Epon and sectioned for LM and EM.

Antidromically identified NSO neurons were either silent or displayed spontaneous firing patterns. We identified immunocytochemically (double-labeled) about 50-75% of LY-injected neurons as neurophysin (NP) containing. UV irradiation (7-15 min) of LY-NP somata resulted in a dark DAB photo-oxidation product (DAB-POP) within each LY marked cell. We find a direct correlation between the intensities of the LY fluorescence and that of DAB-POP within marked neurons. At the ultrastructural level, a fine granular DAB-POP is distributed throughout the cytoplasm of LY-NP neurons. The ultrastructural features of some injected neurons are well preserved and show intact synaptic boutons innervating the DAB-POP filled soma, axon or dendrites. Other injected neurons display swollen mitochondria or endoplasmic reticulum and have electron dense nuclear spots. These results are similar to those we find in goldfish (Reaves et al., Neurosci.Lett. in press '82).

These results suggest the feasibility of morphological (LM/EM) and intracellular electrophysiological analysis of the rat hypothalamo-neurohypophysial explant to model neuroendocrine function. (Support by NIH Grant NS-13411 and a Neurobio. Fellowship to MTL)

148.5 LHRH IN FETAL HYPOTHALAMUS: IN VIVO AND IN VITRO STUDIES. J. Matthews-Bellinger, J.C. King, W.H.J.Douglas* and A.A. Arimura*
Dept. of Anat., Tufts Univ. Sch. of Med., Boston, MA 02111 and Laboratories for Molecular Neuroendocrinology and Diabetes, Depts. of Med. and Anat., Tulane Univ. Sch. of Med., New Orleans, LA 70114.

Dispersed monolayer cultures of neurons offer a simplified yet versatile system to examine modulators of neurosecretion. In contrast to other neuropeptides, LHRH has been particularly difficult to detect within dissociated cultured neurons, although the peptide has been detected by RIA in the medium. Preoptic/hypothalamic regions from 18 day rat fetuses were dissociated either by a modified sieving technique or by mild trypsinization combined with gentle trituration, and plated at $5 - 7 \times 10^4$ viable cells/cm^2 of culture dish. Medium consisted of a 1:1 mixture of Ham's F-12: Dulbecco's MEM supplemented with glucose (to 620 mg%), insulin (20mU/ml), penicillin, streptomycin, glutamine, and 10% fetal calf serum. Non-neuronal cells were allowed to form a sparse background layer, after which further proliferation was suppressed with cytosine arabinoside (2×10^{-6}M) (on day 4 or 5). Neurons attach and extend neurites within 3 to 4 days after plating. Maturing cultures contain a variety of neuronal cell types with soma ranging from 5 to 25 µm in diameter. The majority are bipolar or tripolar with extensive fine neurites typically branching at a distance from the cell body.

Neuropeptide producing cells were identified by immunocytochemistry using the peroxidase-anti-peroxidase method of Sternberger. Cultures were fixed for 15 minutes at 21 °C in 5% acrolein in Sorensen's buffer, washed, then pretreated sequentially with $NaIO_4$, $NaBH_4$, and DMSO. Cultures were incubated for 2 to 3 days at 4°C with primary antisera in the presence of 0.4% triton-X. A small percentage of neurons in these cultures are immunopositive for substance P, somatostatin or tyrosine hydroxylase. In contrast, the majority of neurons are immunoreactive with LHRH antiserum # 422. Reaction is completely abolished when the antiserum is preabsorbed with synthetic LHRH. Immunopositive LHRH is concentrated in the cell body, extending only 10-20 µm along neurites. Antiserum # 422 requires the free N- and C- terminals of LHRH for binding and probably indicates the presence of active decapeptide hormone. In vivo perikaryal LHRH is not revealed with antiserum # 422 either in adult or in day 18 fetal rat brains. However, LHRH is detected in perikarya of both adult and fetal brains with an antiserum that allows modifications of both the N-and C-terminals (#419), as expected for larger precursor forms. A conversion of LHRH molecular species in vitro from an inactive to an active form within perikarya is suggested.

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148.6 PROTOCEREBRAL NEUROENDOCRINE SYSTEM OF THE TOBACCO HORNWORM: MORPHOLOGY AND PHYSIOLOGY OF IDENTIFIABLE NEUROSECRETORY CELLS. Grant M. Carrow*, Ronald L. Calabrese and Carroll M. Williams*. (Spon: Edmund A. Arbas). The Biological Laboratories, Harvard University, Cambridge, MA 02138.

Cell bodies of protocerebral neurosecretory cells (NSC) in isolated, desheathed brains from pupae of the tobacco hornworm, Manduca sexta, were distinguished according to size, position, and reflective opalescence. Individual NSC were stained by intracellular injection of lucifer yellow or horseradish peroxidase. Dendritic arborizations of stained NSC from both lateral and medial groups were concentrated in the cortical region between the groups, a finding which suggests interactions among the cells. Moreover, a medial NSC was found to be dye-coupled at the midline of the brain to an axon collateral of a lateral cell. Branched terminals of the NSC were located in the corpora cardiaca and in the corpora allata, thereby confirming multiple neurohemal sites.

Intracellular penetration of the NSC somata in isolated, desheathed brains revealed resting membrane potentials of -30 to -40 mV, broad (20-40 msec) overshooting action potentials, and postsynaptic potentials. The cells were electrically responsive to depolarizing current injected through the intracellular microelectrode. Replacement of the bathing medium with a high K+ medium also depolarized the cells. Extracellular stimulation of the nerves joining the neurohemal organs to the brain resulted in antidromic impulses recorded in the cell bodies. The pattern of axonal projections determined physiologically agreed with that determined morphologically.

Certain of the cells under study synthesize and release prothoracicotropin (PTTH), a polypeptide hormone which regulates development. Our ongoing research is aimed at the use of the system described here, along with the stimulated release and assay of PTTH (Carrow et al., 1981, PNAS, 78:5866), to identify the PTTH neurosecretory cells and to study their neuronal and hormonal control throughout development.

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148.7 STRUCTURE OF INDIVIDUAL NEUROSECRETORY CELLS OF THE BRAIN IN CRICKETS. M. D. Zaretsky* and W. J. Loher* (SPON: E. Marg). Depts. of Zoology and Entomology, Univ. of California, Berkeley, CA 94720.

Individual neurosecretory cells of the pars intercerebralis of the brain in crickets, Teleogryllus commodus, are shown to have diverse and complex structures when injected with the fluorescent dye Lucifer Yellow (LY) (Stewart, W., Cell, 14: 741, 1978). The action potentials recorded intracellularly with the dye-injecting electrodes are of 7-50 msec duration, typical of insect neurosecretory cells. These experiments indicate there are neurosecretory cells of the pars intercerebralis that do not communicate directly with the organs of the retrocerebral complex. In addition, LY injections reveal additional arborizations and finer details of the structure of neurosecretory cells whose general features have been discovered by cobalt sulfide staining via the nerves to the corpora cardiaca, corpora allata and hypocerebral ganglion (Mason, C. A., Z. Zellforsch., 141: 19, 1973; Koontz, M. and Edwards, J. S., J. Morph., 165: 285, 1980).

The cells of the pars intercerebralis may be grouped according to structure and location of their axons and arborizations. There are cells with axons that cross the midline within the protocerebrum, running ventrally and emerging from the posterior surface of the brain in the nerves of the corpora cardiaca (NCC I), a pathway that has been described by many different histological techniques in several insect groups. Iontophoretic injection of LY demonstrates not only do the axons of these cells branch extensively in the protocerebrum, but also that they arborize in the deutocerebrum and tritocerebrum.

There is a distinct group of cells of the pars intercerebralis that do not have axons that leave the brain. The axons of these cells branch extensively in the protocerebrum and deutocerebrum. A region of prominent, repeated axonal branching is located medially and ventrally near the oesophageal foramen. This area has been described in another species of cricket, Acheta domesticus, as a neurohaemal region, containing neurosecretory fibers (Geldiay, S. and Edwards, J.S., Z. Zellforsch., 145: 1, 1973). These cells also arborize extensively in the more dorsal and lateral regions of the brain. The location of their somata, the extensive branching of their axons in a neurohaemal area and the long durations of their action potentials suggest that the cells of this group are neurosecretory.

148.8 A VISUALLY INDUCED, GABA MEDIATED IPSP IN A CRUSTACEAN NEUROSECRETORY CELL. M. D. Kirk and R. M. Glantz. Dept. of Biology, Rice Univ., Houston, TX 77251.

A discrete group of neurosecretory cells, analogous to the Medulla Externa X-organ of other crustacea, has been found in the crayfish. There are approximately 20 of these cell bodies clustered at the proximal-medial edge of the second optic neuropil of the crayfish eyestalk. The morphology of these neurosecretory cells (obtained from lucifer yellow and HRP injections) is as follows: A short neurite leads from the soma to a profusion of fine dendrites within the medullary neuropil. The dendrites lie in a single vertical plane within the second tangential cell layer of the Medulla. One or two axons originate near the cell body, and project distally terminating proximal to and within the first optic neuropil (L amina).

Using a semi-intact eye-cup preparation (Kirk et al., 1982) intracellular recordings were made from the neurosecretory cell bodies. Action potentials actively invade the soma and are extremely long in duration (½ width = 7 msec). The most remarkable characteristic of the cells is their response to light stimuli directed to the cornea of the eye. They are inhibited by step increases in illumination and the response lasts for the duration of the stimulus. Transient off-inhibition is commonly present. The IPSP reverses near resting membrane potential and appears to be Cl^- dependent. Iontophoresis of gamma-aminobutyric acid (GABA) within the neuropil produces an IPSP with a similar reversal potential and GABA also densensitizes the light response. Release of the neurohormone is proposed to induce dark adaption (and circadian?) migration of visual screening pigments and/or changes in photoreceptor sensitivity, therefore the light induced IPSP provides a pathway for sensory control of neurosecretion.

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148.9 NEUROPEPTIDE SYNTHESIS AND RELEASE IN ISOLATED NEUROSECRETORY CELLS OF THE CRAYFISH. H. Aréchiga, L. Rodríguez-Sosa* and T. de la Vega*. Dept. Physiol., Ctr. Research and Advanced Studies, IPN., Apartado Postal 14-740, México, D.F. 07000.

The eyestalk of crayfish is known to release several neurohormones of peptidic nature. No information is available as to the specific cells related to their synthesis and release. In previous studies (see Aréchiga, H. and Huberman, A. Peptide modulation of neuronal activity in crustaceans. In: The Role of Peptides in Neuronal Function. J.F. Barker and T.G. Smith, Eds. Marcel Dekker, Inc. 1980. p. 317-349), we have characterized a neuropeptide which reduces the excitability in the crustacean nervous system. This neurodepressing hormone (NDH) is mostly located in the eyestalk. This report presents evidences on the location and some properties of the cells of origin of NDH. The experiments were carried out in adult specimens of the crayfish Procambarus clarki and Procambarus bouvieri, of either sex. Intermolt animals were only used. The eyestalks were incubated in a mixture of collagenase and papain, in calcium-free saline solution (Van Harreveld's). Various combinations of enzyme concentrations and incubation times were tested until chosing the most appropriate. Cell disaggregation was followed under microscopic control with a Zeiss inverted microscope. NDH was recovered from the tissue samples by extraction in acetone and chloroform and successive stages of Sephadex G-25 and G-15 columns, followed by paper electrophoresis. Its determination was made by its ability to reduce the tonic firing rate of isolated abdominal stretch receptors of crayfish. Cells recovered after dispersion of various regions of the eyestalk were tested for NDH. This mapping showed that more than 75% of NDH content in cell bodies was in the ventromedial region of the medulla terminalis. Groups of these cells were incubated in high-potassium solutions ranging from 10 to 80 mM. NDH was released in proportion to potassium concentration, up to ~ 80% of the total content of the cells. The amount released increased with time of incubation within a 30 min. range. After depletion, cells were incubated in a mixture of aminoacids (Gibco's MEM) and NDH content was followed by sampling at 10 min. intervals. In 1-2 hours, there was a full recovery of the basal hormone level. This result was not obtained after incubating the cells in saline solution or if protein synthesis inhibitors were added to the aminoacid mixture.

This results indicate that NDH synthesis is confined to a restricted population of neurons in the eyestalk, and that its release may take place outside the terminals, in a place near or at the cell body.

148.10 IN VITRO PEPTIDE SYNTHESIS AND NEUROSECRETION FROM A CRAB NEUROHAEMAL ORGAN. E. Stuenkel* (Spon: I. M. Cooke). Bekesy Laboratory of Neurobiology, Univ. of Hawaii, Honolulu, HI 96822.

^{3}H-labelled peptide secretion from single X-organ-sinus gland (XOSG) preparations was studied. This neurosecretory system has discrete somata (X-organ, XO), axon and terminal (sinus gland, SG) regions. A single XOSG of Cardisoma carnifex was mounted in a double chamber apparatus which permits: 1) separate superfusion of somata and terminals, 2) intracellular electrode recordings from somata and nerve terminals, and 3) extracellular recording of impulse activity in the XOSG tract as it passes the barrier between the chambers. Peptide secretion was stimulated by selective exposure of somata or terminals to a 10 min pulse of saline containing 113 mM K^+ (10XK).

XOSG-specific protein and peptides were radiolabelled in vitro (5 hr pulse : 19 hr chase) with ^{3}H-leucine (130-170 Ci/mM). Peptides produced in the somata and those axonally transported to the terminals were analyzed by SDS-PAGE, HPLC and bioassay. Results indicate that radiolabel in the peptide fraction (10,000 daltons) is associated with at least 5 separate XOSG-specific peptides. Crustacean hyperglycemic hormone(s) and erythrophore concentrating hormone represent 3 or 4 of the peptides radiolabelled. In 5 experiments intracellular terminal records were made during 10XK depolarizing pulses with simultaneous collection of superfusate samples for direct correlation of release from and electrical activity in the nerve terminals. Selective application of 10XK to the SG terminals showed a depolarization, with spiking, lasting approx. 2 min before attainment of a non-spiking plateau 25-30 mV depolarized from rest potential (-20 to -25 mV absolute). An increase in ^{3}H-efflux from the SG terminals appeared in superfusates approx. 2 min subsequent to the application of 10XK to the SG and lasted throughout the exposure to 10XK. No increase in ^{3}H-efflux was seen from the XO somata although simultaneous intracellular recordings from the XO and SG show that depolarization of similar magnitude occurs in both on application of 10XK to the SG. Selective application of 10XK to the XO somata also showed a depolarization, with spiking, to reach a stable level of approx. -20 to -25 mV absolute; simultaneous recording from the SG terminals revealed only a 6-10 mV depolarization. Under these conditions no increase in resting ^{3}H-efflux is seen from either the XO or the SG. In all cases there was recovery to pre-test rest potential, activity and ^{3}H-efflux rates after return to normal saline. The peptide nature of the 10XK-induced increased ^{3}H-efflux from the SG terminals was confirmed by high voltage paper electrophoresis (50-60 V/cm; pH 1.9; 1-2 hr) prior to and subsequent to acid hydrolysis, and by HPLC. Supported by NIH grant NS 15453 to I. M. Cooke.

148.11 PREDOMINANT PEPTIDES IN LYSATES AND PERFUSATES OF THE SINUS GLAND OF CARDISOMA CARNIFEX: ISOLATION AND AMINO ACID COMPOSITIONS. R. Newcomb* (SPON: H. Gillary). Bekesy Laboratory of Neurobiology, University of Hawaii, Honolulu, Hawaii 96822.

The predominant peptides of a major invertebrate neurosecretory structure, the crustacean sinus gland, have been purified by reversed phase liquid chromatography (RPLC) from the crab Cardisoma carnifex. Peptides were characterized by 1) RPLC elution properties relative to known peptides, 2) amino acid composition, 3) bioassay and 4) elution properties of their fluorescamine derivitives. Nine peptides were constantly present in large quantity and several other peptides were present in variable and/or lesser quantity (approx. 10 µg total peptides/sinus gland). All of these peptides were unique to the sinus gland and not found in the surrounding nervous tissue. Based on the amino acid compositions, the peptides were divided into three distinct groups. One set of eight peptides is explicable as variable cleavage fragments from one or several parent peptides of M.W. ca. 3000 daltons. The amino acid compositions of these peptides are not identical to any previously published for sinus gland peptides. Another set of three distinct peptides have amino acid compositions similar to those published by Keller (J. comp. Physiol. 141, 445) for the hyperglycemic hormone of various species, and at least two of these show hyperglycemic activity. An erythrophore concentrating hormone of the crab was shown to be indistinguishable from that of the shrimp (Fernlund, B.B.A. 371, 304) both in its RPLC elution properties, and in its absorbance and fluorescence characteristics. Experiments in which the peptides and amino acids in perfusates of the sinus gland were analyzed as fluorescent derivatives demonstrated that the majority of these peptides (about 2 pmol or 1% of each peptide per sinus gland in 10 min) were released by depolarization with 10X K^+. There was no release of peptides observed in zero Ca^{++}, normal or 10% hypoosmotic normal salines, and peptide release in 10X K^+-zero Ca^{++} perfusates was variable relative to normal 10X K^+ perfusates. A small and variable quantity of amino acids (<1% of total sinus gland amino acids) was released by depolarization by the 10X K^+ and a large amount of amino acids (about 20% of total sinus gland amino acids) was released over a 50 min zero Ca^{++} perfusion. Thus, peptide release was specific in the sense that it was independent of amino acid release and was also not due to the decrease in $[Na^+]$ in the 10X K^+ perfusion. The characterization of the peptides in the sinus gland has allowed the study of the significance of the proteolytic processing of small peptides at the sinus gland nerve terminals as related to the final forms released. Supported by NIH grant NS 15453 to I. M. Cooke.

148.12 MONENSIN-INDUCED CHANGES IN BAG CELL ULTRASTRUCTURE: RELATION TO IMPAIRED PRODUCTION OF NEUROSECRETORY PEPTIDES. Michael E. Yates and Robert W. Berry. Dept. of Cell Biol. and Anat., Northwestern Univ. Med. School, Chicago, IL 60611.

Treatment of various mammalian cell types with monensin produces marked dilation of the Golgi apparatus and impairs the release of secretory proteins (Tartakoff & Vassalli, J. Exp. Med. 146, 1332-1345, 1977). In the bag cells of Aplysia californica, 10uM monensin produces a dramatic impairment of processing of precursor and intermediate proteins which give rise to the neurosecretory peptides AP and ELH (Yates & Berry, Soc. Neurosci. Abst., 1981). We have therefore performed an ultrastructural study of bag cells exposed to monensin in order to determine whether the biochemical blockades could be due to changes in Golgi apparatus function arising from disruption of its structural integrity.

Bag cell organs of Aplysia were exposed to 10uM monensin in artificial seawater and nutrients (experimental) or were incubated in seawater and nutrients alone (control) for 6 or 16hr. The organs were fixed in 3% glutaraldehyde for 4hr, postfixed in 1% OsO_4 (0.1M phosphate buffer) for 1.5hr, dehydrated, and embedded. Electron micrographs revealed the presence of large vacuoles and an absence of "normal" Golgi apparatus in experimental cells at both time points. Control cells contained Golgi profiles with classical morphological characteristics, while the large vacuoles present in monensin-treated cells were absent. The ultrastructure of all other observed organelles appeared identical in control and experimental cells. The vacuoles in bag cells exposed to monensin for 16hr often contained a single, round, electron dense aggregate of material closely associated with the vacuole membrane at a single point. Furthermore, terminal varicosities of rough endoplasmic reticulum (RER) containing similar electron dense cores were often present at 16hr. Neither of these structures were apparent in control cells. We conclude that the impaired processing of the precursor and blockade of subsequent cleavages is very likely due to the effects of monensin on the Golgi apparatus. It is probable that a disruption of formation of Golgi-derived vesicles is responsible for the blockade of late steps in the processing sequence. The apparent self-association of electron dense material in dilated Golgi elements indicates that this material does not require a limiting membrane to form a dense core. The association of the core with the membrane of these vacuoles may represent a secretory protein-membrane protein interaction allowing for segregation of material as it exits the Golgi apparatus. The dense core material in the terminal varicosities of the RER may be self-associated precursor protein.
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149.1 SYSTEM IDENTIFICATION OF HUMAN TRICEPS SURAE STRETCH REFLEX. R.E. Kearney, I.W. Hunter and P.L. Weiss. Biomedical Engineering Unit, Faculty of Medicine, McGill University, Canada, H3G 1Y6.

The interpretation of stretch-evoked reflex responses is complicated by the fact that the observed pattern will depend upon both the underlying reflex mechanisms and the time course of the stretch used to evoke the response. The objective of the present study was to use engineering systems analysis techniques to identify the dynamics of the human triceps surae (TS) stretch reflex in terms of its impulse response by deconvolving the position input from the observed response.

Subjects were instructed to maintain a tonic contraction at a level ranging from 0-20% of the maximum voluntary contraction. Their ankles were then driven according to a repeated, computer-generated, stochastic pattern with a peak-to-peak amplitude which varied from 0.025 to 0.25 rad. Position, torque and smoothed, rectified surface EMG's were recorded and ensemble averaged over 25 stimulus presentations.

Impulse response functions identified between ankle velocity and TS EMG accounted for about 60% of the observed EMG variance. However, the impulse responses were noisy and the predicted EMG was systematically smaller than the observed EMG during the dorsiflexing phases of displacement and larger than predicted EMG during the plantarflexing phases.

These findings suggested that a direction dependent nonlinearity might be present. Consequently, we computed the impulse responses relating half-wave rectified velocity to TS EMG and found that the resulting impulse responses were less noisy and accounted for significantly more variance (mean 70%) than did those relating ankle velocity to TS EMG.

These uni-directionally rate sensitive impulse response functions were dominated by a large peak at about 40 ms followed by a smaller period of reduced activity. This is consistent with its mediation by spindle primary afferents. Although the shape of the impulse response remained unchanged, its amplitude, which provides a measure of relative gain, varied systematically with the level of contraction and the displacement amplitude. Multiple regression analysis demonstrated that most of the variation in the impulse response amplitude could be attributed to a linear increase with level of contraction (measured by average EMG) and a linear decrease with displacement amplitude.

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149.2 PHASE LEAD OF EXTENSOR EMG ON WRIST FLEXION DURING LIGHTLY-DAMPED OSCILLATIONS, POSTURAL TREMOR, AND PHYSIOLOGICAL ACTION TREMOR OF THE HAND. R. N. Stiles. Department of Physiology and Biophysics, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

A major question in motor control is whether neural feedback normally accentuates or attenuates resonant neuromuscularlimb oscillations. The purpose of this study was to quantify relative neural feedback phase for resonant hand oscillations obtained under three different conditions. System damping was also quantified under conditions for which this damping is positive, i.e., for lightly-damped oscillations.

Using a small accelerometer, lightly-damped oscillations, postural tremors and physiological action tremors were detected from the unsupported hand of normal human subjects. Damped oscillations (DOs) were produced by tapping the subject's hand at a rate of about 1/sec. Bipolar surface EMGs were detected from two wrist extensors. Raw EMGs were digitized, full-wave rectified and smoothed, producing amplitude-demodulated EMGs. The relative phase between highly-correlated EMG modulation and the negative of acceleration (i.e., hand position) of the resonant hand oscillations was obtained by cross-spectral analysis. Damping ratios were calculated for different peaks of the DOs for DO frequencies between 4 and 9 Hz. EMG-wrist flexion phase was calculated for both normal (physiological) and enhanced (large-amplitude) postural and action tremors.

Results show that: (1) For damped oscillations, reflex damping increased (became more positive) with increased phase lead of extensor EMG signal on wrist flexion. (2) There was a larger phase lead of EMG on wrist flexion during physiological action tremor than during normal postural hand tremor. This phase advance was associated with an increased displacement amplitude of the action tremor relative to that of the postural tremor. (3) The phase lead of extensor EMG on wrist flexion decreased with increased displacement amplitude of either postural or action tremor of the hand.

These results suggest that feedback damping of resonant hand oscillations may normally increase during times of voluntary hand movement as a result of a mechanism that advances the phase of the neural feedback signal on wrist flexion. Results also suggest that tremor amplitude may be enhanced above normal levels as a result of decreased reflex damping. (Supported by USPHS Grant NS 14730.)

149.3 THE DETECTION OF SYNCHRONY USING RECTIFIED NEUROGRAMS AND ELECTROMYOGRAMS: CONSIDERATIONS WITH RESPECT TO SPIKE-TRIGGERED AVERAGING. T.M. Hamm. Dept. of Physiol., Univ. of Arizona Health Sci. Ctr., Tucson, 85724.

A test for synchrony between the reference event (an afferent or motor unit action potential) in a spike-triggered average (STA) and other (non-reference) events has been employed in which the area of an averaged, rectified neurogram or electromyogram (EMG) is compared to the area of the corresponding average of the high-pass filtered neurographic (or EMG) signal (Milner-Brown et al, J. Physiol. (Lond.)228:285-306,1973; Roscoe et al, Proc. Int. Union Physiol. Sci. XIV:227-228,1980). This test has been demonstrated to be a sensitive measure of afferent and motor unit synchrony (Roscoe et al, 1980), although its dependence on the signal-to-noise ratio (S/N) of the reference event and on the S/N and variance band (the distribution of times of occurrence with respect to the reference) of non-reference events poses limitations on its use and interpretation. This report provides a further analysis of the effects of these factors on this synchronization test. In particular, the test's ability to detect multiple events which are 'loosely' correlated with the reference has been investigated. It has been argued that such events would contribute significantly to a postsynaptic potential obtained by STA (Kirkwood and Sears, J. Physiol. (Lond.)322:287-314, 1982).

Simulations were performed in which the probability density functions of signals with several correlated events and the expected values of those signals after rectification were calculated. These simulations yield the relations between the increase in signal variance in the presence of correlated events, which is responsible for the increased area of the rectified average, and the S/N and variance band of such events. The number of correlated events and the probability of discharge near the reference event were shown to be interchangeable in that a number of non-reference events with low probabilities of discharge make a contribution to the rectified average which is approximately equal to that of a single event with a correspondingly higher probability.

These results indicate that this test for synchrony is capable of detecting multiple events which are loosely correlated. Moreover, the critical role of variance suggests a test for synchrony in which the variances of reference-triggered and randomly-triggered signals are compared. Such a test would be independent of effects of the reference event's S/N.

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149.4 CAN THE ELECTRICAL THRESHOLD OF GROUP Ia & Ib AXONS BE REVERSED IN HUMAN SUBJECTS? C.J. Heckman*, S.M. Condon*, R.M. Enoka, and R.S. Hutton. Dept. of Kinesiology, Univ. of Washington, Seattle, WA 98195.

After prolonged vibration of the Achilles tendon in cats, the electrical threshold of group Ia fibers is increased above that of Ib afferents (Coppin et al., J. Physiol., 210:18, 1970). Ia receptor threshold to stretch activation remains unchanged (Fetz et al., J. Physiol., 293:173, 1979). The possibility of similar findings in human (N=4) soleus muscle was investigated indirectly by alternately measuring (EMG) tendon tap (T) and Hoffmann (H) reflexes before and after 20 minutes of vibration (100Hz) applied to the Achilles tendon. The foot was strapped at a 90° ankle angle. Reflexes were elicited and vibration was applied while the subjects maintained a constant low-level plantar flexion static force (~86N) under conditions of visual monitoring of force feedback. The inter-stimulus interval (ISI) per reflex type was 10 seconds and the ISI between alternate reflex stimuli was 5 seconds. Percent change in intra-reflex magnitudes between control and post-vibration conditions was calculated using the average of 16 responses elicited over 160 seconds during each recording sequence.

In all subjects, immediately after vibration, H-reflexes were either abolished or markedly reduced as compared to control mean responses. In contrast, T-reflexes initially showed moderate to marked enhancement above control levels. The time course of recovery for the post-vibration H-wave responses varied across subjects but H-waves remained depressed in 3 of the 4 subjects for greater than 35 minutes. T-waves returned to control values within 15-30 minutes. Three of the 4 subjects were re-tested and these findings were replicated. Similar decrements in the H-reflex or enhancement in the T-reflex could not be induced by 20 minutes of sustained static force alone. The depth of H-reflex depression appeared to parallel the magnitude of the soleus tonic vibration reflex and the degree of perceived sensory illusion of foot movement (usually into dorsiflexion) during vibration.

In re-testing another subject, H-reflexes were elicited alternately at 1.2 and 1.8 X threshold (Th). Initial post-vibration H-waves at 1.2 X Th were negligible (<5% of control) but were present (83% of control) at 1.8 X Th. M-waves were still present before and after vibration as were T-reflexes. These findings were also replicated in another previously tested subject. In all, the results are in accord with the predicted outcome on spinal reflexes using the Coppin et al. paradigm and offer indirect evidence that similar reversals in group Ia electrical thresholds above Ib axons might be produced in humans. Further experimentation using, for example, microneurographic recording techniques appears warranted.

149.5 TOPOGRAPHIC WEIGHTING OF HOMONYMOUS SINGLE IA AFFERENT INPUT TO MEDIAL GASTROCNEMIUS MOTONEURONS. Sylvia M. Lucas, Tim C. Cope and Marc D. Binder. Dept. of Physiol. & Biophys., Univ. of Washington, Sch. of Med., Seattle, WA. 98195.

Last year we reported that homonymous group Ia input to cat medial gastrocnemius (MG) motoneurons is "topographically weighted" in that afferents innervating a given intramuscular compartment provide relatively stronger synaptic input to motoneurons innervating the same compartment than to motoneurons innervating other compartments within the muscle (Lucas and Binder, Neurosci. Abstr. 7: 561, 1981). We have subsequently performed a series of experiments to determine whether differences in Ia projection frequency and/or average EPSP amplitude underlie this topographic weighting. In 10 barbiturate-anesthetized cats we have studied 186 single Ia afferent-motoneuronal connections using the spike-triggered averaging technique. The motoneurons were classified as either "same branch" or "other branch" depending upon whether the Ia afferent and motor axon were contained in the same or different intramuscular nerve branches. No difference was found in the connectivity of Ia afferents to the "same branch" and "other branch" motoneurons (95%, N=102 vs 94%, N=84, respectively). However, the mean EPSP amplitude was larger in the "same branch"(92±8 μV; N=97) than in "other branch" (77±7 μV; N=79) motoneurons. This difference was most striking (statistically significant, $p<0.05$) with putative large motoneurons (rheobase ≥ 10 nA), for which the mean "same branch" EPSP was 82±12 μV (N=48) while that in the "other branch" cells was 52±5 μV (N=37). In 60 cases it was possible to compare the EPSPs produced by a "same branch" afferent and an "other branch" afferent in the same motoneuron. The mean ratio of the "same branch" to "other branch" EPSPs was 1.7, which was both statistically significant and consistent with the value of 1.8, which we had previously found for aggregate EPSPs. Again the weighting appeared strongest in putative large motoneurons (ratio = 2.0; N=27). These results suggest that the topographic weighting of homonymous Ia afferent input to cat medial gastrocnemius motoneurons is mediated by a gradient of synaptic strength rather than by a gradient of connectivity.

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149.6 Mechanical Properties of the Primate Forelimb during Imposed Displacement. F. Lenz*, S. Schloegel*, W. Tatton, R. Tasker*, (Spon. J.Wojtowicz), Playfair Neurosci. Unit, Univ. of Toronto, Toronto, Canada.

It has been proposed that the function of the stretch reflex is to improve the linearity or 'spring-like' properties of muscle (Nichols and Houk, J. Neurophys. 39(1976) 119). We have computed mechanical properties of primate forelimb muscle-joint systems as a step in understanding how reflexes might function in the control of the whole limb.

Torque motor imposed extensions of the metacarpophalangeal joint, the wrist and the elbow in squirrel monkeys evokes reflex electromyographic (EMG) activity in the flexor muscles acting across these joints. Joint angle, EMG response and tension were measured; acceleration and velocity were calculated from position records by numerical differentiation. Since not all trials evoked a reflex response, trials were separated according to the the presence or absence of an EMG reflex response and averaged as separate groups (termed reflex and non-reflex trials). The tension (corrected for inertial effects) and displacement at the end of limb segment distal to the joint were used to estimate average stiffness of the joint for reflex and non-reflex trials.

At each joint studied, the positional dependence of tension was significantly less linear (as judged by regression analysis) for non-reflex trials than for reflex trials. 'Non-reflex stiffness' was significantly greater in distal than in proximal joints but 'reflex stiffness' values for the three joints were not significantly different. Both measures of stiffness were independent of velocity. The inertial moments of the three limb segments varied by three orders of magnitude. Over similar ranges of baseline EMG activity and velocity, the reflex EMG response, expressed as a fraction of the maximium response to electrical stimulation of the motor nerve, was four times less in distal flexors than in elbow flexors. Therefore reflex activity appears to compensate for differences in the mechanical properties of different muscle-joint systems to produce a linear displacement-tension relation characterized by similar average stiffness across each of three contiguous joints in the primate forelimb. (Supported by: TGH Found., Ont. Heart Found., MS Soc. of Canada, MRC MA5218)

149.7 SPECTRAL ANALYSIS OF CROSSED EXTENSOR CONTRACTIONS IN THE DECEREBRATE CAT. S. A. Burgstahler* and E. K. Stauffer. Sch. of Med., Univ. Minn., Duluth, MN 55812.

When the peroneal nerve is stimulated in a decerebrate cat, reflex contraction of the contralateral extensor muscles is produced in the hindlimb. Superimposed on the contractions are small amplitude oscillations detectable in high gain force and acceleration recordings from (1) isolated muscles and (2) the intact limb. The purpose of this study was to determine the frequency and amplitude characteristics of the contracting plantaris muscle during the crossed extension (X-EXT) reflex. These findings were then compared with stretch-evoked oscillations observed in intact limbs.

Experiments were performed on eleven cats decerebrated at the midcollicular level. In eight cats, the left hindlimb was denervated except for the nerve supplying plantaris. Records of muscle force, acceleration, and electromyographic (EMG) activity were obtained before, during, and after stimulation (100 Hz, 10-15 sec) of the contralateral peroneal nerve. Following a series of X-EXT reflexes, dorsal roots L_7- S_2were severed and the reflex was evoked again. In the remaining cats, limbs were kept intact with oscillations recorded during X-EXT reflexes and following a quick dorsiflexion-release of the foot. Spectral density estimates of filtered force (3-30 Hz) and acceleration were calculated from isometric and anisometric data respectively.

Isolated plantaris muscles oscillated within a broad range of frequencies, 6-25 Hz. There was no significant change ($p> 0.80$) in the frequency of oscillation before (11.9 ± 2.5* Hz) versus after section of the dorsal roots (11.6 ± 1.4 Hz), but amplitude decreased significantly (0.8 ± 0.2 g vs 0.2 ± 0.1 g respectively, $p< 0.02$). To date our results from anisometric preparations with intact hindlimbs show a frequency of 14.5 ± 0.5 Hz and an acceleration of 0.5 ± 0.3 cm/sec^2 following crossed extensor stimulation. This is in contrast to anisometric recordings of an isolated plantaris muscle which oscillated at 19.0 ± 1.5 Hz with an amplitude of 159.3 ± 24.9 cm/sec^2. Anisometric recordings of stretch-evoked clonus from intact hindlimbs gave a frequency of 12.4 ± 0.4 Hz at an acceleration of 12.7 ± 4.9 cm/sec^2. These findings are consistent with a "final common pathway" whose input signal has a baseline frequency that tends to remain constant but whose amplitude can be altered by inputs from other sources. The observed changes of the pathway's output signal can be explained by changes of the external load.

*All values are $\bar{X}$ ± S.E.M.

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149.8 Differences between the crossed extension reflex and the flexion reflex in the decerebrate cat. A.M. Moudy* and J.A. McMillan (SPON: D.E. Phillips). Biology Department, Montana State Univ., Bozeman, MT 59717

A widely-accepted tenet of spinal cord physiology is that the crossed extension reflex (CER) is a crossed component of the ipsilateral flexion reflex (FR) and, as such, is mediated by interneurons in the FR pathway. In this report we provide evidence to suggest that the CER may in fact be independent of the FR in some important respects.

Experiments were performed on young adult cats. Initial surgical procedures, including a midcollicular decerebration, were carried out under ketamine anesthesia. Indwelling stimulating electrodes were placed in the left sciatic nerve. Isometric tension was recorded simulataneously from the left semitendinosus (FR) and the right quadriceps femoris group (CER).

The CER consistently demonstrated prolonged central summation, or "windup", at frequencies of stimulation lower than those which evoked such summation of the FR. In some cases the CER developed "reflex tetanus" at frequencies producing much less obvious summation of the FR. Such observations do not support the concept that interneurons which mediate the ipsilateral FR are also directly responsible for mediation of the crossed CER.

In three experiments it was possible to evoke the CER with stimuli below the threshold necessary to evoke the FR. Even though these were not consistent observations, they still suggest that the CER is not mediated solely by primary afferents and interneurons which first evoke the FR.

Previous work in our laboratory has pointed out that the CER is affected differently than is the FR by descending influences from suprasegmental and supraspinal inputs (McMillan and Koebbe, Exp'l. Neurol., 73:233-242, 1981). The results presented in the present report are consistent with these earlier findings in that they emphasize some important differences between the CER and the FR.

In conclusion, we propose that the relationship between the CER and the FR is not as intimately interdependent as is classically considered.

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149.9 IDENTIFICATION OF SKELETOFUSIMOTOR ACTION IN REFLEXIVELY ACTIVATED MUSCLE Stephen E. Grill*, W. Zev Rymer, Corey L. Cleland Neuroscience Program and Physiology Dept., Northwestern Univ. Med. Sch., Chicago, IL, 60611

The existence of skeletofusimotoneurons (β) has now been directly documented with electrophysiological techniques in reduced preparations. Recordings from muscle spindle afferents in reflexively active muscles of decerebrate cats have been used to derive several indirect criteria for skeletofusimotor action. Here we describe the results of experiments in which these criteria were tested directly by examining the fusimotor input of characterized spindle afferents.

The criteria used to recognize β-activity on spindles rely on the finding that γ-motoneurons are activated well below extrafusal threshold, whereas β-motoneurons are activated above extrafusal threshold (presumably along with α's). Therefore, we attribute fusimotor effects occuring well above extrafusal threshold to β-fibers. We compared apparent fusimotor effects on spindle afferents at various levels of EMG and force, varying the level of motor output by invoking the crossed extension reflex (CER). Thirty-five spindle afferents have been classified to date as to showing fusimotor effects above extrafusal threshold. Thirty-seven percent (13/35) showed static fusimotor effects during hold phases of ramp stretches, 48% (14/29) showed static fusimotor effects during ramp releases, 29% (10/35) showed dynamic fusimotor effects during ramp stretches, and 66% (23/35) increased their rates in parallel with tension under isometric conditions.

After afferent discharge patterns were characterized, the cats were anesthetized, S1 and L7 ventral roots were sectioned and small fascicles were tetanically stimulated. Direct evidence for β-input onto muscle spindles was obtained if increasing the rate of stimulation of efferent filaments above extrafusal fusion frequency resulted in increasing discharge of the spindle afferents. In this way we showed that 7 of the 10 afferents studied received at least 1 β-fiber, 4 received 2, and 2 received 3. We found 7 β-statics and 8 β-dynamics. Two of the 3 afferents for which we could not demonstrate β-innervation showed no fusimotor effects above extrafusal threshold during the physiological characterization. One afferent that received 2 β-fibers did not display fusimotor effects above extrafusal threshold.

Our data suggest that spindle afferents that show fusimotor effects above extrafusal threshold are usually innervated by skeletofusimotor fibers. β-fibers may extend the range of fusimotor action to cover the full range of motor output.

149.10 Identification of Spinal Interneurons Mediating the Clasp-Knife Reflex in the Cat
Corey L. Cleland, W. Zev Rymer, Stephen E. Grill* Neuroscience Program and Physiology Dept., Northwestern Univ. Med. Sch., Chicago, IL 60611

The clasp-knife reflex, first described in spastic human patients, occurs in decerebrated cats that have had their dorsolateral spinal funiculi sectioned. The reflex consists of powerful and sustained inhibition of homonymous motor output when an extensor muscle is stretched beyond a certain length. Contrary to earlier beliefs, group III and IV muscle afferents are probably responsible. We have identified a population of spinal interneurons that we believe mediate the clasp-knife reflex in the cat. Previously, we demonstrated that these interneurons are potently excited by the same muscle afferents that generate the clasp-knife reflex and that the static and dynamic input-output properties of the interneurons are similar to those of the clasp-knife reflex. In this communication we report that these interneurons inhibit extensor motoneurons and that their activity is closely correlated with decreases in emg during the clasp-knife reflex.

These interneurons were shown to inhibit MG motoneurons by using spike-triggered averaging to correlate the extracellularly recorded activity of interneurons and the intracellularly recorded membrane potential of extensor motoneurons. In 4 chloralose anaesthetized and spinalized cats we found that 3 pairs produced an average with a prominent hyperpolarization 1-3 ms following the occurrance of the interneuronal action potential.

Direct correlations between interneuronal activity and motor output were obtained by simultaneously recording interneuronal activity and the emg from the soleus muscle. In 7 unanaesthetized but decerebrated and dorsal hemisectioned cats, all 6 interneurons showed a time course of activity paralleling the time course of inhibition of the emg.

Taken together, our studies provide 3 lines of evidence that these interneurons mediate the clasp-knife reflex. First, the interneurons have the appropriate afferent and efferent connections. Second, the static and dynamic input-output properties of the interneurons and the clasp-knife reflex are similar. Finally, the activity of the interneurons shows a tight and undissociable correlation to clasp-knife inhibition.

149.11 LOW FORCE FEEDBACK GAIN IN THE STRETCH REFLEX OF THE DECEREBRATE CAT FOLLOWING DORSAL HEMISECTION OF THE SPINAL CORD, J.J. Cath* and P.E. Crago (Spon: J.T. Mortimer). Applied Neural Control Lab., Case Western Reserve University, Cleveland, Oh. 44106.

Decreases in stretch reflex stiffness have been observed following dorsal hemisection of the spinal cord in decerebrate cats. It has been postulated that the lesion would interrupt descending inhibitory projections to tendon organ pathways and that this release would increase force feedback gain and thus decrease stiffness. This hypothesis was tested by measuring force and EMG responses to imposed length disturbances before and after a reduction in muscle gain. EMG responses remained the same in the face of drastic reductions in muscle force, implying that the gain of force feedback is low in these preparations.

Cats were anesthetized with halothane and nitrous oxide and a midcollicular decerebration was performed by aspiration. The soleus muscle was dissected and connected to a force transducer mounted on a length servomechanism. Anesthesia was then discontinued. EMG was recorded from fine intramuscular wire electrodes and was processed by full wave rectification and low pass filtering.

The stiffness of the stretch reflex was evaluated by recording a series of responses to ramp and hold stretches at various initial forces and initial lengths prior to spinal cord hemisection. In all cases, initial force was graded by crossed extensor reflexes. A dorsal hemisection of the cord at T9-10 was then performed under light anesthesia. A series of responses was measured at a nearly constant initial force with the initial length adjusted to prevent the appearance of the clasp-knife reflex. These responses served as the controls for estimating the gain of force feedback from tendon organs.

Muscle gain was reduced by intravenous administration (2 mg/Kg) of dantrolene sodium. Finally, a series of responses was recorded with length disturbances identical to those designated above as controls, but at an initial force that resulted in the same value of initial EMG as was recorded in the controls. Ensemble averages of the responses in each series were constructed.

The initial force and the change in force due to the length disturbance were typically half of the values measured prior to muscle gain reduction. If force feedback was contributing to the stretch reflex, the EMG response should have been greater after the drug administration since the autogenetic inhibition would have been lower. However, the two EMG responses were virtually identical, implying that the gain of force feedback was negligible and probably no different from the values reported by others for the decerebrate cat with an intact spinal cord.

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149.12 NEONATAL STRETCH REFLEXES, G. L. Gottlieb, B. M. Myklebust, G. C. Agarwal and R. D. Penn Dept. of Physiology, Rush Med. Ctr., Chicago, IL 60612.

Previous studies have demonstrated that in the normal adult, activation of the soleus muscle (SOL) by rapid mechanical stretch, tapping the SOL tendon, or electrical stimulation of the tibial nerve (H-reflex) evokes SOL EMG activity at monosynaptic latencies (30-50 ms), while the shortening antagonist tibialis anterior muscle (TA) is electrically quiet [1]. In contrast, in patients with spasticity due to a perinatal insult (ie, cerebral palsy (CP)), activation of SOL evokes simultaneous EMG activity of comparable magnitudes and equal latencies in TA and SOL. We have termed this phenomenon "reciprocal excitation" to contrast it with the normal spinal cord circuitry of reciprocal inhibition [2,3]. These findings are suggestive of a developmental error in the CP spinal cord, in addition to the brain insult. To quantify the magnitude of reciprocal excitation in CP we have computed the "TA:SOL reflex ratio" by measuring the ratio of the peak-to-peak amplitudes of the stretch-evoked EMG, 30-50 ms after the tap.

To investigate the normal development of the reflex ratio, preliminary studies have been performed on normal neonates (1-10 days) and a small number of children up to 6 years old. Surface EMG activity from TA and SOL was recorded when tendon taps were delivered to SOL and TA tendons.

Neonates demonstrate a TA:SOL reflex ratio which is larger than in the normal adult but smaller and more variable than in CP. This ratio decreases with age, and by 6 years it is in the range of the normal adult.

The normal neonate's spinal cord shows hyperexcitability with respect to the normal adult's, which apparently decreases during normal development. The relative contributions of spinal cord hyperexcitability and reciprocal exciation to the elevated neonatal TA:SOL ratios we have observed are under investigation.

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This work was supported in part by NIH Grants NS12877 and NS15630

149.13 *TACTILE PLACING IN FORELIMB AND HINDLIMB OF NORMAL AND SPINALIZED KITTENS.* *N.S. Bradley*, J.L. Smith and J.R. Villablanca. Depts. Kinesiology & Ment. Ret. Res. Cntr., UCLA, Los Angeles, CA 90024*

Early work by Bard led to the conclusion that tactile placing (TP) is a cortically dependent reflex. Forrsberg, et al. (Acta Physiol. Scand. 92:114, 1974) however, have reported the presence of TP in the hindlimbs (HL) of spinalized kittens. Preliminary testing in our laboratory of cats 8-9 months of age, spinalized (T-12) at 2 or 12 weeks of age, failed to elicit HL placement. We thus decided to quantify the time course and characteristics of TP development in normal and spinalized kittens to determine if behaviors are similar.

Tactile placing was tested from 3-62 days of age in 15 kittens from 3 litters. Spinal transections (T-12) were performed in 9 kittens at 14-16 days of age. All kittens were tested for forelimb (FL) dorsal, lateral, and medial TP, and the HL tested for dorsal TP with the hip joint at 90°. Additionally, spinalized cats were tested for HL-TP with the hip joint in further extension (120°). Onset, duration and force of contact were measured by a force transducer attached to the triggering stimulus. Latency of placement was recorded by downward displacement of an adjacent horizontal plate overlying a second transducer.

Normal kittens rarely demonstrated FL-TP before the 3rd week (0-8% of trials) or HL-TP before the 5th week (0-17% of trials). By the 4th week, dorsal FL-TP occurred 32% of trials with an average latency of 1113 ms. By the 5-6th week, HL-TP occurred 21% of trials with an average latency of 1143 ms. Therefore, there was a gradual increase in frequency and a decrease in latencies of TP response over the two month period suggesting an ongoing maturational process (Villablanca & Olmstead, Develop. Psychobiol. 12: 101, 1979). Additionally, responses of normals were noted to be discrete movements of small amplitude. Spinal kittens demonstrated similar responses in FLs, but not in HLs. In only 10 of 675 HL trials (1.5%) over the post-transection period observed did the paw contact the horizontal plate without associated airstepping or other reflex augmenting movements. Of the 10 trials, average placement latency was 912 ms. Typically, HL contact triggered abrupt withdrawal, airstepping, and banging of the paw against the vertical wall housing the contact transducer. Thus, the HL behaviors of normal and spinalized kittens differed in both contextually associated behaviors and probability of occurrence.

Results suggest that tactile placing does not develop in spinalized kittens. Those HL responses interpreted by others as TP in spinalized cats may be tactile-triggered airstepping or withdrawal responses. We conclude that tactile placing requires the involvement of supraspinal centers.

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149.14 EFFECTS OF DORSAL RHIZOTOMY ON DOG FEMORAL CONDYLAR CARTILAGE. B.L. O'Connor*, M. Palmoski*, K.D. Brandt*. (SPON: J. DiMicco) Depts. Anat. and Med., Indiana Univ. Sch. Med., Indianapolis, IN 46223.

Neuropathic arthropathies are complications of diabetes mellitus, tabes dorsalis, syringomyelia, leprosy, and other disorders that affect the peripheral and central nervous system. The joint lesions resemble an exaggerated form of osteoarthritis and are generally thought to result from a breakdown of protective muscular reflexes owing to decreased or abolished sensory input. In the absence of such reflexes, the joint may be subjected to increased microtrauma during day-to-day activities, the cumulative effect of which is the formation of severe osteoarthropathy.

We tested this hypothesis by performing dorsal rhizotomies (L4 through S1, inclusive) to desensitize the left hind limbs of dogs that were then permitted to exercise ab lib in roomy pens for up to 16 months. Appropriate sham operated control animals were also prepared. Prior to sacrifice, patellar reflexes were tested to verify their absence in the experimental operated animals and their presence in sham operated animals. Spinal cords of the sacrificed animals were examined histologically using the Kluver-Barrera stain, and showed dorsal column degeneration in the experimental animals but not in the sham operated controls.

The articular cartilage of the femoral condyle of the left (operated) knees and the right (non-operated controls) were examined for biochemical (water and uronic acid content, and 35S uptake for newly synthesized proteoglycans), histological (Safranin-O affinity, fibrillation, cloning, hyper- or hypocellularity, tidemark invasion by blood vessels, subchondral bone cysts) and gross evidence of osteoarthritis.

No abnormalities were identified in any of the joints examined. The water content of operated and nonoperated knee cartilage was about 70% in both the experimental and sham rhizotomized animals. No statistically significant difference in 35S incorporation or uronic acid content was observed between operated and nonoperated sides in either group. Both the gross and histological appearance of the cartilage were normal.

It is concluded that complete desensitization of a limb plus ad lib exercise will not necessarily result in a Charcot joint, and that some additional factor, possibly an acute joint injury, is required to initiate the development of neuropathic arthropathies. Supported by PHS Grant AM26951 to B.L. O'Connor.

149.15 THE PAIRED H-REFLEX AND ITS CORRELATION WITH EEG COHERENCE AND ACADEMIC PERFORMANCE IN NORMAL SUBJECTS PRACTICING MEDITATION. R. K. Wallace, P. J. Mills*, D.W. Orme-Johnson , M. C. Dillbeck*, and E. Jacobe*. Dept. of Biology, Maharishi Intern. Univ., Fairfield, IA 52556.

Studies using the paired H-reflex to investigate the neurophysiological effects of meditation (TM) have reported: 1) a decrease in both the amplitude of the activity of the frontalis muscle and in the amplitude of the H-reflex during meditation (Chenard,1982), 2) a significant correlation between flexible performance on a concept learning task and paired H-reflex and frontal EEG coherence (Dillbeck et.al.,1981), and 3) a significant correlation between paired H-reflex recovery, high alpha coherence and creativity (Haynes et.al.,1977). In addition, we noted a longitudinal facilitation at intervals 100, 150, 200,and 250 msec. in experimental males practicing an advanced TM program, while the experimental females and controls showed no significant change (Wallace et.al.,1979).

In our present investigation we have replicated previous research correlating EEG parameters and paired H-reflex recovery and further noted a significant correlation between paired H-reflex recovery and academic performance in subjects practicing meditation. The subjects were 13 male (mean age 22 years, mean length TM practice, 48 months) and 9 female (mean age 21 years, mean length TM practice 45 months) healthy university students. The paired H-reflex correlated significantly with EEG alpha and theta coherence at intervals 100-1000 msec.. The H-reflex correlated with academic performance (student grade point average) at intervals 150-500 msec.. The H-reflex did not correlate with SAT scores or WAIS IQ.

Although the area is not well understood, previous research has found the H-reflex to be sensitive to changes in states of awareness. For example, Van Boxtel (1976) showed that a constant alpha index is accompanied by stable H-reflex amplitudes and a decreasing alpha index (indicative of drowsiness) is accompanied by decreasing H-reflex amplitudes. Pivak and Mercier (1979) showed that during nonrapid eye movement (NREM) sleep (stages 2 and 4) the H-reflex facilitation period was significantly reduced. The part of the H-reflex recovery curve most sensitive to changes in states of awareness is the facilitation period of 100-300 msec. (Gassell,1970). This period also most highly correlated with the EEG parameters and academic performance. These findings suggest that the H-reflex, in particular the facilitation period of the recovery response, may be an indicator of awareness or wakefulness level. Whether the results reported here are unique to participants of the TM program is unknown. Further research is necessary to extend these findings to a larger and more general population.

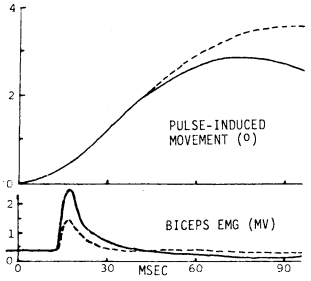
149.16 Learned Change in the Spinal Stretch Reflex: Effects on Movement and Antagonist Muscle Behavior. D.J. Braitman, J.R. Wolpaw, V.A. Kieffer*. (SPON: K.D. Barron). Arm. For. Radiobio. Res. Inst., Bethesda, Md. 20014 & Center Labs & Res., NYS Dept. Hlth, & Depts. Neurol. & Anat., Albany Med. Coll., Albany, N.Y., 12201.

Monkeys can gradually change spinal stretch reflex (SSR) amplitude (amp) without change in initial muscle length or background EMG (Wolpaw et al. NS Abst.7:249(1981)). We studied concurrent changes in movement and antagonist muscle behavior.

Five monkeys learned to keep elbow angle at 90° (±1.5°) against steady extension force. If this angle was held for a randomly selected 1-2s period, and if the average absolute value of biceps EMG (from chronic i.m. electrodes) for the final 0.2s was 1.0-1.5 X a set value, a brief force pulse extended the elbow 3-4°. SSR amp was measured as the average absolute value of biceps EMG 12.5-21.5ms after pulse onset minus background EMG amp. Under the Control condition, reward occurred at 100ms. Under the SSR↑ or SSR↓ condition, reward occurred only if SSR amp was more (SSR↑) or less (SSR↓) than a criterion value. Control condition data were obtained for 10-50 days. SSR amp was stable over this period. Then the animal was exposed to the SSR↑ and SSR↓ conditions in succession for prolonged periods. With imposition of each condition, SSR amp changed appropriately over 10-20 days (Wolpaw & Seegal, this vol.).

The first 40ms of pulse-induced movement did not change with SSR amp. After 40ms, movement was inversely related to SSR amp (Fig.). This 20-25 msec delay between the SSR and its apparent effect on movement was presumably the time necessary for muscle contraction following excitation.

Triceps EMG was minimal under all three conditions. Thus the antagonist does not appear to play a major role in SSR amp change or the accompanying post-40ms change in movement.



Pulse-induced movement and biceps EMG amplitude from one animal. Solid lines are a day's data (>3,000 trials) under the SSR↑ condition; dashed lines, a day's data under the SSR↓ condition. Note that background (pre-pulse) EMG and the first 40 msec of movement are the same for the two days.

149.17 CHARACTERISTICS OF M SPIKES IN CAT MOTONEURONS AND THEIR SIGNIFICANCE FOR THE MEASUREMENT OF SMALL COMPOSITE Ia EPSPs.

R.M. Reinking*, T.M. Hamm, B.R. Botterman and D.G. Stuart.
Dept. of Physiol., Univ. of Arizona Health Sci.Ctr., Tucson 85724.

Some characteristics of intracellularly recorded M spikes in cat motoneurons have been determined in order to evaluate techniques for subtracting this spike from recordings of homonymous monosynaptic composite Ia EPSPs evoked by stimulation of intramuscular nerve branches (viz., Neuroscience Letters 24: 35-41, 1981).

Antidromically conducted M spikes were recorded in 44 hyperpolarized hamstring motoneurons in three deeply anesthetized cats with sectioned dorsal roots. The decay of the M spike was characterized by multiple components, resulting in the M spike being larger at late times than has been supposed. Comparison of the longest time constant of the M spike with that of the motoneuron membrane and of the time course of the M spike with the results of a simulation indicate that the time course can be attributed to the passive electrical properties of the cell.

Averaging normalized, individual M spike records yielded a composite M spike for use in the measurement of Ia EPSPs ("subtraction method"). This method was compared to previous methods requiring extrapolation of the M spike ("extrapolation method") or recording EPSP waveforms above and below the M spike threshold ("addition method"). Estimates of EPSP amplitude (in mV) by these methods in 19 motoneurons gave the following results:

Subtraction Method	Addition Method	Extrapolation Method
2.84 ± 1.75	2.82 ± 1.88	3.45 ± 1.73

Comparison of the subtraction and extrapolation methods in a larger number of cells (n=117) gave similar results.

A comparison of EPSP estimates by the subtraction method with the measured EPSP value in cells (n=12) in which the maximum EPSP was attained at a stimulus strength subthreshold to that of the M spike gave the following results:

EPSP Amplitude	Estimated EPSP	Difference
2.84 ± 1.61	2.90 ± 1.55	-0.06 ± 0.30

These results indicate that the M spike makes a significant contribution to the apparent amplitude of small, composite Ia EPSPs and that accurate estimates of EPSP amplitude in the presence of an M spike can be obtained by the subtraction or the addition method, while the extrapolation method results in an average overestimation of 0.3 - 0.6 mV.

Supported by USPHS grants NS 07888, RR 05675, HL 07249, NS 05871 and NS 06462. Present address of B.R. Botterman: Dept. of Cell Biology, Univ. of Texas Health Sciences Center, Dallas, TX 75235.

150.1 SUPERFICIAL AND DEEP AFFERENT INPUTS TO SINGLE NEURONES IN THE SENSORY AND MOTOR CORTEX OF MACACA FASCICULARIS. M. Sirisko* and B.J. Sessle (SPON: A.T. Storey). Faculty of Dentistry, University of Toronto, Toronto, Canada M5G 1G6.

The activity of single neurones in the face motor cortex and adjacent sensory cortex was recorded extracellularly in ten barbiturate-anaesthetized monkeys in which continuous monitoring was made of blood pressure, expired CO_2 and rectal temperature. Afferent inputs to cortical neurones were tested by the use of electrical stimulation of the exposed facial, hypoglossal and temporalis muscle afferent nerves, the oral mucosa and facial skin, and by the use of natural (tactile, pressure, jaw and tongue stretch) stimuli applied to the orofacial region. Neurones projecting directly to the brainstem were identified by their antidromic response to stimulation of the facial, hypoglossal or trigeminal motor nucleus. In eight of these same animals, the motor cortex representation of the face, tongue and jaw had previously been determined in the unanaesthetized state by a systematic intracortical microstimulation examination of evoked muscle activity. Histological verification was made of selected microelectrode tracks.

Single and multi-unit antidromic activity evoked from the brainstem motor nuclei could be recorded from the precentral cortex within the confines of the motor cortex as defined by microstimulation. Afferent inputs were tested in 60 of these corticobulbar projection neurones (estimated mean conduction velocity of 20 m/sec): 20% of these neurones had a deep (muscle afferent) input; none were found with a superficial (cutaneous or oral afferent) input. The other major population of neurones (n=197) recorded comprised non-projection neurones that received superficial and/or deep inputs. Thirty-seven neurones excited only by cutaneous or oral afferent stimuli had latencies of 4-8 msec and were confined primarily to postcentral areas 1 and 3b. Most of the 133 neurones activated exclusively by one or more of the deep inputs had latencies and thresholds indicative of Group I or II muscle afferent excitation; although some were found in areas 4 and 3b, these neurones were predominantly confined to area 3a, and spontaneously discharged with a characteristic bursting pattern. Neurones with convergent superficial and deep inputs (n=27) were primarily located postcentrally, in area 3b. These findings indicate that in the anaesthetized monkey, deep inputs from facial, tongue and jaw muscles predominantly pass to neurones in area 3a of the sensorimotor cortex and that more caudally located neurones receive superficial or convergent afferent inputs.

Supported by the Canadian Medical Research Council.

150.2 PARALLEL MODULATION OF FORELIMB STRETCH REFLEX AND SCALP RECORDED EVOKED POTENTIALS, D.J. Crammond* and W.A. MacKay, Department of Physiology, University of Toronto, Canada, M5S 1A8.

Although in nonhuman primates the components potentially involved in a long latency transcortical reflex loop are well studied, there is yet a paucity of information from human experiments or indications as to the functional operation of such a system. In an effort to evaluate this further in humans, subjects were seated with right arm raised to shoulder level and elbow resting at 90° in the manipulandum of a DC torque motor. The signal to the motor produced a 70 msec ramp torque maintained for a further 50 msec, in either the flexion or extension direction. The subject either reacted to the torque perturbation to oppose the displacement or remained totally passive. Trials were repeated every 2 sec. The EMG from both triceps and brachialis muscles was rectified and integrated and scalp recorded perturbation evoked potentials (EP's) were recorded at a gain of 74,000: bandwidth D.C. to 1 KHz. The EP signal was recorded over C3 on the 10.20 system for placement (the contralateral forearm somatic sensory cortex), with reference to the linked mastoid processes. The EP, EMG's arm position and torque measured at the wrist were collected for each trial and averaged on a PDP-11 computer, the number of averages varying from at least 300 up. As a control the procedure was repeated with the subject's arm resting over but not stimulated by the manipulandum.

In the passive condition there was very little or no reflex EMG recorded. In the EP, a negative wave often began 60 to 70 msec after the onset of the torque perturbation. A late positive peak of about 300 msec was seen in some subjects. In the react condition, late stretch reflexes appeared in the EMG record. The EMG onset latencies varied from approximately 40-70 msec depending on the subject or muscle group studied. However, in the EP, a clear divergence from the control or passive condition was always evident at 60-70 msec when a consistently positive plateau developed lasting for up to 50 msec and often producing a positive D.C. shift for several hundred milliseconds on top of which late positive components at 200 msec may be superimposed. Prior to the divergence at 60-70 msec, the EP trace was identical in the react and passive conditions. The results show that there is a different cortical response to the forelimb perturbation depending on the subject's reaction. Regarding a central contribution to late, long loop reflex EMG components, a cortical divergence of response patterns at 60-70 msec indicates that the early EMG reflex components in the react condition (40-70 msec) are probably not transcortical reflexes although descending cortical signals could influence their modulation at other levels. However, late EMG components starting at about 70-80 msec could involve modifiable transcortical loops. Supported by the MRC of Canada.

150.3 CORTICAL CELL RESPONSE TO UNLOADING DURING A MAINTAINED POSTURE. E.M. Schmidt, J.S. McIntosh, and L.L. Glenn. Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Many precentral neurons have intense and prolonged discharges during accurate small movements Fromm & Evarts (Neurosci. Lett. 5:259, 1977). Evarts & Fromm (Neurosci. Lett. 5:267, 1977) suggested that the intense discharge of these neurons during small movements might be due, in part, to sensory input. They tested this hypothesis by applying torque pulses to a manipulandum at various times during the task. However, the movements induced by the pulses were large compared to the small accurate movements (5° pulse as compared to about a 1° movement) and may have provided considerably more sensory input to the neurons than was present with small movements. To circumvent this, we tested the hypothesis with smaller torques that unloaded rather than stretched the active muscles.

A monkey (Macaca mulatta) was trained to perform a wrist flexion-extension task (±30°) against a simulated spring load (.136 NM/Rad) produced by a torque motor. The hand was in an open position and coupled to the torque motor by a form fitting rubber mold, in an open position, to eliminate finger movements during the task. The monkey was required to maintain wrist flexion or extension for a random period (1.0 to 1.25s) within alternating target zones 12° in width. At the end of the hold period, a 30ms torque pulse was introduced that unloaded the contracting muscles and resulted in an approximately a 1.5° wrist movement.

Single cell activity was recorded in the arm area of the precentral cortex. Discharge rates for 100 units were recorded; 70 were well-related to the task. This sample was composed of 28 pyramidal tract neurons (PTNs) and 42 non-PTNs. Of the PTNs, 54% (15) responded to the unloading pulse and 43% (18) of the non-PTNs responded. Each group of neurons was subdivided into flexor or extensor units depending on the phase of the movement in which they were active. The discharge of the majority of neurons (23 of 33) was inhibited during the unloading, starting at latencies between 5 and 25ms (12.3 mean). The largest single grouping of cells exhibited inhibition when the active movement was unloaded. This response is consistent with Evarts' findings and suggests that these neurons are recipients of sensory inputs that may be part of a long-loop reflex which helps stabilize the position of the wrist.

150.4 SENSORY RESPONSES OF PYRAMIDAL TRACT NEURONS IN UNANESTHETIZED RHESUS MONKEY. Steven P. Wise, Christoph Fromm* and Edward V. Evarts. Laboratory of Neurophysiology, N.I.M.H., Bethesda, Md. 20205.

Pyramidal tract neurons (PTNs) were identified in precentral motor cortex (MI) and postcentral somatic sensory cortex (PoC) of monkeys trained to pronate and supinate the forearm. 199 PTNs in MI and 72 PoC PTNs were isolated in the present study. Every putative PTN was tested by the spike collision method and no unit was accepted if it failed to show the predicted collision properties. In MI 74% of the task-related PTNs responded to the limb displacements, while in PoC 85% did so. PTN responses to passive ramp-and-hold displacements of the forearm were examined and fell into three categories: transient, sustained and combined transient-sustained.

Larger PTNs (those with shorter antidromic latencies) tended to exhibit transient responses to passive limb displacement. Conversely, smaller PTNs more frequently showed sustained responses. The mean antidromic latency of MI PTNs that showed only transient responses was 1.5 ± 0.9 ms while those that showed only sustained responses (2.9 ± 1.6 ms) or combined transient-sustained responses (2.5 ± 1.2 ms) had longer antidromic latencies. PoC PTNs had generally longer antidromic latencies than MI PTNs, but a similar pattern was observed: smaller PTNs were more likely to show sustained responses. The effect of electrode sampling bias towards larger PTNs exaggerates the contribution of transient-only responses to the total PTN sample. By applying the correction function of Humphrey and Corrie (J. Neurophysiol. 41: 216, 1978) for microelectrode sampling bias it can be seen that the vast majority (64%) of MI PTNs which have sensory responses to limb displacements show sustained responses.

This finding offers clues concerning the functional significance of feedback to somatic sensorimotor cortex during voluntary movement and shows that a substantial population of MI PTNs receive continuous feedback during posture as well as during the dynamic phase of movement.

150.5 MOTOR-DEPENDENT NEURONAL ACTIVITY IN PREMOTOR AND MOTOR CORTEX FOLLOWING VISUOSPATIAL CUES. M. Weinrich, K.-H. Mauritz* and S.P. Wise. Lab of Neurophysiology, NIMH, Bethesda, MD 20205

"Responses" of neurons in the premotor and motor cortex to visual stimuli have been reported. To investigate the contingency of these activity patterns on planned movements we have recorded the activity of single neurons in these cortical fields of two unanesthetized rhesus monkeys while they performed a motor task. A key feature of the task is that the monkeys were presented with physically identical visual stimuli in two conditions: one requiring a movement, and the other requiring the monkeys to withhold a movement.

Monkeys were seated in a primate chair facing a tangent screen. A "position" spot and a "target" spot were projected on the screen; the monkeys were otherwise in darkness. Their left arms were enclosed in a plastic brace which allowed motion at the elbow. A potentiometer measured the angle around the elbow and controlled the "position" spot. The "target" spot was controlled by a computer. First, the animals were required to align the two spots for 1.3 sec. Next, in 5/6 of the trials, termed "go" trials, the computer would move the "target" to one of five randomly chosen locations on the screen and simultaneously brighten it. The animals had to maintain their original position for a randomly varied delay (0.8-2.4 sec), after which the target dimmed to its original luminance. This dimming was the cue for the animals to realign the spots within 1.2 sec. For the remaining "no-go" trials, the computer brightened the target without moving it. After a randomly varied delay (0.8-2.4 sec) the light dimmed. The monkeys had to remain in their original positions throughout the period when the target was brightened.

Activity following the brightening of the target was of two types. 198 neurons responded transiently to the target jump. The mean peak activity of these transients was approximately the same for the "no-go" trials as in the trials requiring a movement. 150 neurons with sustained activity following the visual cue were recorded. 80% of these neurons had greater activity during "go" trials, and the mean activity during "go" trials was greater than twice the activity during "no-go" trials. These results suggest that some aspects of sustained activity elicited by visual stimuli from premotor and motor cortex neurons may be contingent on planned motor acts.

150.6 RECIPROCAL SUPPRESSION OF ANTAGONIST MUSCLE ACTIVITY BY SINGLE CORTICOMOTONEURONAL CELLS IN THE PRIMATE. R.J. Kasser* and P.D. Cheney. Departments of Anatomy and Physiology, University of Kansas Medical Center, Kansas City, Kansas, 66103.

Motor cortex cells which are functionally coupled to motoneurons of muscles coactivated with the cell during voluntary movement (agonist muscles) may be identified in awake monkeys by the appearance of a transient postspike facilitation in spike-triggered averages of rectified EMG activity. Since these cells are most likely monosynaptically connected to motoneurons of facilitated muscles, they have been referred to as corticomotoneuronal (CM) cells (Fetz & Cheney, J. Neurophysiol., 44: 751, 1980). We wished to test whether such cells, in addition to facilitating one or more agonist muscles, also simultaneously inhibit the antagonist muscles. Rhesus monkeys were trained to make wrist movements alternating between two target zones - one in flexion and the other in extension. EMGs from 4-6 forearm flexor and extensor muscles were recorded differentially with pairs of multistranded stainless steel wires inserted pericutaneously. CM cells are usually tonically active during only the agonist phase of an alternating movement and become inactive during the antagonist phase. To maintain CM cell activity throughout the entire wrist movement cycle, glutamate was iontophoresed through one barrel of a double-barreled microelectrode while simultaneously recording cell discharge through a tungsten microelectrode contained within the second barrel. Glutamate iontophoresis currents ranging from 70 to 321 nA (mean 190 nA) increased the activity of motor cortex cells an average of 34 Hz during the agonist phase of movement and 27 Hz during the antagonist phase without significantly altering the detailed structure of the response pattern during movement. Functional effects of 20 wrist movement related motor cortex cells on flexor and extensor wrist muscles were tested by computing spike-triggered averages separately for each set of muscles. Nine of these cells had no effect on either flexor or extensor muscles despite a strong correlation between the cell's activity and wrist movement. Of the remaining cells, five produced postspike facilitation in one or more of the agonist muscles and postspike suppression in one or more antagonist muscles. Five cells produced only postspike facilitation of agonist muscles and had no effect on the antagonists. One cell produced only postspike suppression of antagonist muscles and had no effect on agonists. In conclusion, our results suggest that some motor cortex cells, like muscle spindle Ia afferents, have reciprocally organized connections with flexor and extensor motoneuron pools. (Supported by NIH grant NS16262).

150.7 PT STIMULATION PRODUCES CONDUCTANCE DECREASE IPSPs IN NEURONS OF THE MOTOR CORTEX OF CATS. L. Bindman*, C. Woody, E. Gruen*, and R. Betts* (SPON: R. Pay). Depts. of Anatomy and Psychiatry, UCLA Medical Center, Los Angeles, CA 90024.

Effects of pyramidal tract (PT) stimulation at the level of the facial nucleus (100 μs pulse of up to 10 mA or train of 2 to 6 pulses 10 ms apart, delivered every sec) were studied in intracellular recordings from 62 cells of the motor cortex of awake cats. Technical procedures have been described elsewhere (Woody and Black-Cleworth, J. Neurophysiol., 1973; Tzebelikos and Woody, Brain Res. Bull., 1979). Of these cells 10 showed an IPSP that decreased with hyperpolarization and, in 5 of the ten cells, the IPSP was reversed with additional hyperpolarizing current. In 9 of the cells, it was possible to measure a decrease in resistance at the time of the IPSP. This IPSP has been recognized previously by other investigators and is thought to reflect an increase in chloride conductance.

In 30 of the remaining cells, a quite different IPSP was found within the same 35-120 msec period following PT stimulation. In each of these cells, the IPSP increased in size with the application of hyperpolarizing current and could not be reversed with hyperpolarization. With depolarizing current the IPSP decreased in size. The input resistance of 8 of these cells was determined by computer analysis and showed an increase in resistance during the IPSP. This was done by comparing the magnitude of a continuously repeated (20 ms on, 20 ms off) bridge pulse during the IPSP with that prior to the PT shock that elicited the IPSP. Analysis of six additional cells showed a more complex conductance change with a hump of increased resistance at the peak of the IPSP appearing within a broader trough of decreased resistance. Conductance decrease IPSPs have been reported previously (Siggins et al, Science, 1971; Engberg and Marshall, Acta. Physiol. Scand., 1971; Smith and Weight, Nature, 1977), but not in neurons of the motor cortex. Conductance decrease IPSPs were seen in these cells irrespective of whether antidromic spikes were produced by PT stimulation. (Supp. by BNS 78-24146, HD 05958, and AFOSR 81-0179).

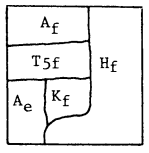
150.8 RESPONSES OF RAT CEREBRAL CORTEX TO MEDULLARY PYRAMIDAL TRACT STIMULATION. T. A. Harrison and A. L. Towe. Dept. of Physiol. & Biophys., Univ. of Washington Sch. of Med., Seattle, WA 98195.

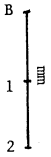
The major component of the response in the cerebral cortex of anesthetized rats to medullary pyramidal stimulation is a large, short-latency, short-duration, surface-positive potential thought to result from antidromic invasion of PT neurons. Because published accounts contain indications that this response may not be purely antidromic, a study was undertaken to further characterize this response and to determine the manner in which it is produced. As in previous studies, this response (here called the A component) was recorded over most of the parietal and frontal cortex. Its initial and peak latencies varied with recording position; it occasionally exceeded 1.0 mV in peak amplitude. At many recording sites, a small, surface-positive component could be seen preceding the main A component; it showed no latency variation with recording position, and never exceeded 0.1 mV in amplitude. The A component was more widely distributed than this small, early component, attaining its maximal amplitude in contralateral hindpaw primary cortex, with a secondary maximum in contralateral forepaw primary cortex. It extended caudolaterally into somatosensory area II, apparently forming a separate, smaller amplitude distribution. Latency and amplitude decreased with iterative stimulus rates above 50 Hz. The A component disappeared soon after a KCl-induced spreading depression developed, but recovered more rapidly than did the primary response to contralateral forepaw stimulation. Intravenous injection of strychnine sulfate increased the A amplitude to 130-140% of control, whereas the primary response more than doubled in amplitude. Laminar recordings revealed little or no change in the A component down to deep layer III; below that level, the A component rapidly reversed in polarity, becoming a large negative response below layer V. These properties, associated with synaptic rather than purely antidromic responses, are the same as those of the r wave found in the woodchuck and also present in the slow loris and opossum. A similar component has been observed in the rabbit and phalanger, but is absent in the cat, raccoon and macaque monkey. It is concluded that the A component in the rat is an r wave and that it is synaptically mediated by activation of pyramidal tract collaterals, probably within the cortex. It is suggested that the small, early, surface-positive component that is often obscured by the large r wave, and that shows characteristics associated with purely antidromic events, is the true antidromic response. [Supported by USPHS grants NS05136 and 1T32-NS07097]

150.9 INTERNAL ORGANIZATION OF THE HINDLIMB AREA OF THE RAT MOTOR CORTEX. C.F. Sievert* and E.J. Neafsey (SPON: G.C. Gaik). Dept. of Anatomy, Loyola University Stritch School of Medicine, Maywood, Illinois 60153.

Although a hindlimb area in rat motor cortex has been previously described (Hall and Lindholm, Brain Research, vol. 66, 1974), the detailed internal organization of this region was unknown. The present study investigated the internal organization of the hindlimb area by mapping movements elicited by intracortical microstimulation (.25 msec pulses, 10-100 μamps, 350 Hz, 300 msec trains) in the motor cortex of adult Long-Evans rats. The rats were anesthetized with Ketamine HCl (100 mg/kg, IP) and placed in a sterotaxic frame. A small craniotomy was made and a glass insulated tungsten microelectrode (80-120 μ exposed at tip) was inserted perpendicular to the cortical surface to a depth of 1.7 mm. In each rat 30-50 penetrations spaced approximately .5 mm apart were made in an area extending from 1 mm rostral to bregma 4 mm caudally, and from 1 mm off the midline 3 mm laterally. Stimulation currents at each point were initially 100 μamps and were then reduced gradually to threshold (the lowest current reliably evoking a movement).

The drawing below depicts the typical size, location and internal organization of the hindlimb area as determined thus far in 4 rats. Bregma is denoted by B. The movements elicited included hip flexion, H_f; knee flexion, K_f; ankle flexion, A_f; ankle extension, A_e; and toe five flexion, T_{5f}. Not all movements were found in each animal, but the results in each animal were close to that illustrated. Just anterior to the hindlimb area trunk and forelimb movements were found, while posteriorly tail movements were seen occasionally.





Following HRP injections into the lumbar enlargement, a large patch of labeled neurons was found in the hindlimb area as defined by electrical stimulation in the same animal. It is interesting to note that after HRP injections into the cervical enlargement there was a large patch of lightly labeled cells in the hip area of the hindlimb motor cortex. This labeling may be due to collateral branches to the cervical enlargement from fibers projecting to the lumbar enlargement as described by Shinoda in the cat. These collaterals may be functional in control of coordinated hindlimb and forelimb movements.

Supported by NIH grant NS 16146 and BRSG RR 05368 from Loyola Univ.

150.10 THE MOTOR CORTEX OF THE RAT: CONNECTIVITY AND SINGLE UNIT DISCHARGE DURING MOVEMENT. J. P. Donoghue. Lab. of Neurophysiology, NIMH, Bethesda, MD 20205.

The motor cortex (MI) of the rat consists of two cytoarchitectonic areas: an agranular area, called the lateral agranular field (AG_1) and an area with a granular layer IV, which overlaps part of the first somatic sensory (SI) cortex (Donoghue and Wise, Neurosci. Abs. 7:18, 1981). However, the role of these areas in the control of movement is not understood. In the present study, the sources of inputs to AG_1 were identified with axonal pathway tracing methods and, the discharge pattern of single units in this subdivision of the rat MI cortex during motor activity was recorded.

Following injections of horseradish peroxidase restricted to AG_1, labeled cells are found ipsilaterally in the frontal agranular cortex rostral to AG_1, the SI cortex, and the second somatic sensory area. Labeled cells in SI are predominantly in the dysgranular areas. Each ipsilateral projection arises primarily from cells in layers III and V, but a few labeled cells are present in layers II and VI. Commissural inputs to AG_1 arise from cells in layers II-VI of the contralateral AG_1. Thalamic inputs to AG_1 arise from the ventrolateral complex and the ventromedial, central lateral, and posterior nuclei.

The activity of single units in MI and SI cortex was studied in behaving rats trained to repeatedly press a fixed bar with their forelimb. Water reinforcement was given after forelimb force exceeded a set level. Unit discharge in the forelimb region of AG_1 was related to changes in forelimb force. One group of units in AG_1 increased discharge rate up to 100ms prior to a change in force and peak discharge in these cells was as much as 50 impulses per second over background. Another group of AG_1 units decreased discharge before or during force changes. Both groups of units were located near sites where intracortical microstimulation at 30 uA or less produced movements of the forelimb. In addition, units in this region appeared to be activated by input from noncutaneous receptors. Units in the forelimb area of SI cortex, where cells are activated by light tactile stimuli to the forelimb, often showed a burst of activity when the rat's paw made contact with the bar.

These studies show that the lateral agranular field of rat MI cortex receives convergent inputs from: (1) two cortical areas involved in sensory information processing; (2) a rostromedial agranular cortical field; (3) contralateral MI; and (4) thalamic nuclei known to receive input from subcortical motor centers. This multiplicity of sensory and motor inputs and the early modulation of unit discharge prior to force changes suggest that neurons in AG_1 are important in the processing and execution of central and peripherally guided motor programs.

150.11 IS THERE A VESTIBULAR INPUT TO THE RAT CEREBRAL CORTEX ? S.E. Knowles, J.K. Chapin and D.J. Woodward. Dept. Cell Biology, Univ. of Texas Health Science Center, Dallas, Texas, 75235.

The aim of this study was to determine if neural projections from the vestibular nuclei of the rat are relayed to the neocortex. In particular we wished to determine whether: 1) cortical area(s) receive vestibular input; 2) these vestibular areas are associated with other modalities; and 3) the thalamic relay nuclei might be involved in this system. Both anatomical and electrophysiological techniques were used to examine these pathways.

Iontophoretic injections of HRP containing 1-3% L-lysophosphatidyl choline were made at various cortical and thalamic sites. HRP injections into areas including ventrobasal (VB) thalamus resulted in retrogradely labelled neurons in the contralateral vestibular nuclei. This same region of VB thalamus could be retrogradely labelled by HRP injections placed in the central SI dysgranular zone (DZ). This zone is situated in SI between the forepaw and head representation spanning approximately 2 square mm. DZ is unresponsive to light cutaneous touch of the periphery in the anesthetized animal, but exhibits deep and cutaneous receptive fields in the awake animal. Cytoarchitechtonically, DZ lacks a thick layer IV and is thus readily discernable by light microscope.

Single unit recordings were obtained from neurons in the DZ of halothane anesthetized rats. Histogram analysis showed evidence of excitation following electrical stimulation of the medial or superior vestibular nuclei (single pulse or train of 3, 0.2 msec width, 1.5 msec separation, 0.2-0.7 mAmp, 0.5-1 Hz) with latencies ranging from 8-30 msec. Excitatory neuronal responses have been recorded in deep layers of the cortex (>1 mm below pial surface) which were frequently followed by a trough of inhibition (lasting up to 70 msec).

In summary, the present results indicate that a portion of the dysgranular zone (DZ) of primary somatic sensory cortex of rat may receive a vestibular input. This input may converge upon cells with deep and/or cutaneous receptive field properties in the awake animal. The ventrobasal thalamus is a likely relay nucleus in this projection which may play a role in motor coordination.

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150.12 CORTICAL AFFERENTS TO THE VIBRISSAE MOTOR CORTEX IN THE RAT. R.R. Terreberry* and E.J. Neafsey. (SPON: F.W. LaVelle). Dept. of Anatomy, Loyola University Stritch School of Medicine, Maywood, IL 60153.

A recent microstimulation study from our laboratory has reported that the vibrissae area in the rat motor cortex is organized into discrete zones controlling either the ipsilateral or contralateral vibrissae (Terreberry and Neafsey, Anat. Rec. 202: 189A, 1982). The present study investigated the cortical afferent projections to the vibrissae motor cortex by mapping retrogradely labeled cortical neurons following injections of horseradish peroxidase conjugated with wheat germ agglutin (HRP-WGA) into the vibrissae motor cortex. The rats were anesthetized with Ketamine HCl (100 mg/kg,IP) and placed in a stereotaxic frame. The cisterna magna was opened to prevent cortical swelling and a small piece of bone (2x5 mm) was removed just rostral to bregma on one side. The vibrissae motor cortex was identified by locating the region where intracortical microstimulation (.25 msec pulses, 5-100 μamps, 350 Hz, 300 msec trains) evoked either ipsilateral or contralateral vibrissae movements. Then .02-.03 μl of a 2% solution of HRP-WGA (Sigma) in physiological saline was injected into the vibrissae motor cortex using a 1 μl Hamilton syringe fitted with a 50 μm diameter tip. After survival periods of 2-2½ days, the animals were reanesthetized, transcardially perfused with 1.25% glutaraldehyde and 1% paraformaldehyde, and the tissue was processed for HRP histochemistry according to the TMB procedure of Mesulam (J. Histochem. Cytochem. 26: 106-117, 1978).

Retrogradely labeled neurons were found in several cortical areas, including the ipsilateral primary vibrissae somatosensory cortex (SI), bilaterally in the secondary somatosensory cortex (SII) and in the homotypic areas of the contralateral primary motor cortex (MI). Positively labeled cells in SI were organized into two different patterns. One pattern consisted of labeled neurons in both the supragranular (layers II,III) and the infragranular (layer V) layers of SI cortex. This pattern of labelling was found at both the medial and lateral borders of the vibrissae SI where there appeared to be "breaks" or "gaps" between the granule cell aggregates of layer IV. The second type of labelling pattern consisted of a narrow band of labeled cells in the infragranular layer V only. This band was not continuous but was broken up into clusters of labeled cells that may correspond with the vibrissae "barrels" of SI. This pattern was found in the intermediate portion of vibrissae SI, not at the borders. No positively labeled cells were found in the contralateral SI. Positively labeled cells in SII were located in layers II,III and V with the ipsilateral SII showing more label than the contralateral SII.

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150.13 A COMPARISON OF THALAMIC AFFERENTS TO THE ROSTRAL AND CAUDAL FORELIMB REGIONS OF RAT MOTOR CORTEX. E. Luke Bold*and E.J. Neafsey. Dept. of Anatomy, Loyola University Stritch School of Medicine, Maywood, IL 60153.

Two separate forelimb motor areas have recently been described in rat frontal cortex (Neafsey and Sievert, Brain Res. 232, 1982). The present study investigated the organization of thalamocortical projections to these functionally similar, yet spatially separated cortical areas by mapping retrogradely labeled thalamic neurons following injections of HRP or HRP conjugated with wheat germ agglutinin (WGA-HRP) into either the rostral forelimb region (RFL) or the caudal forelimb region (CFL). Under Ketamine anesthesia, the RFL or CFL regions were identified by locating the points where intracortical microstimulation (.25msec. pulses, 100uamps or less, 300 msec. train @ 350 Hz) evoked digit or wrist movements rostral or caudal to an area where neck movements were elicited. Then .01-.02 ul of a 30% HRP solution (Sigma VI) or a 5% solution of WGA-HRP (Polysciences and Sigma) in physiological saline was injected into the physiologically defined RFL or CFL using a 1 ul Hamilton syringe fitted with a 50 um diameter pipette tip (typical diameter of injection site= 1.5 mm). After survival periods of 1 to 2 1/2 days, the animals were reanesthetized, transcardially perfused with 1.25% glutaraldehyde and 1% paraformaldehyde and the tissue processed for HRP histochemistry according to the TMB procedure of Mesulam (J. Histochem. Cytochem. 26, 1978).

Following both injections, retrogradely labeled neurons were found in the same basic set of thalamic nuclei which included the ventromedial (VM), ventrolateral (VL), intralaminar, and posterior (PO) nuclei. A few labeled cells were found within the ventrobasal complex (VB) only following large CFL injections but were never seen following RFL injections. Also, RFL injections consistently labeled cells in the paralamellar mediodorsal nucleus (MDpl) whereas such labeling was not observed following CFL injections.

The basic similarity of thalamocortical projections to the two forelimb areas suggests that both are subdivisions of primary motor cortex, a notion also supported by the low microstimulation thresholds found in both areas. The significant labeling in PO is of special interest because some lemniscal afferents to the thalamus terminate in this nucleus (Lund and Webster, JCN 136, 1967), suggesting that PO may transmit peripheral sensory information directly to motor cortex. It is tempting to speculate that this part of PO in the rat may be the homolog of the primate VPLo nucleus since both nuclei are located dorsal to VB and caudal to VL and both PO and VPLo project to layer III of motor cortex (Herkenham, Science 207, 1980; Friedman and Jones, J. Neurophysiol. 45, 1981).

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150.14 COMPONENTS OF THE NEUROMAGNETIC RESPONSE OF THE HUMAN SENSORIMOTOR CORTEX. F. Richer, R. Johnson* and J. Beatty. Department of Psychology, University of California, Los Angeles, CA 90024.

Using a superconductive magnetometer with a 1-cm. resolution, evoked magnetic fields can be recorded from the scalp of normal humans above the sensorimotor cortex during a simple reaction time task.

The movement-related field is recorded for the 500 msecs preceding and following simple finger flexions executed in response to a brief 800 Hz tone using the onset of the flexor EMG activity as temporal reference. Average evoked fields are computed from about 120 finger flexions at each of 24 scalp locations covering an area of 50 cm^2 centered at C3 in the EEG derivations. The field reverses polarity along the axis connecting C3 to the vertex and the point of polarity reversal, which indicates the location of the current source producing the field, is in close agreement with the location of the finger representation on the sensorimotor cortex as determined by somatosensory evoked fields. The amplitude distribution of the different components of the neuromagnetic response reflect current sources orthogonal to the Rolandic fissure and tangential to the surface of the scalp. A depth triangulation procedure is used to confirm the cortical nature of the sources and to determine their depth with a precision of a few millimiters. Both pre-EMG and post-EMG components of the field could be identified.

The precise localization of these independent components as well as their relative sensitivity to movement force and movement perturbations is being investigated.

151.1 NEUROPHYSIOLOGICAL STUDIES OF THE VENTRAL LATERAL GENICULATE-SUPRACHIASMATIC NUCLEUS PROJECTION IN THE RAT. G. A. Groos* and B. Rusak. Physiology Laboratory, Univ. of Leiden, The Netherlands.

The hypothalamic suprachiasmatic nuclei (SCN) are involved in the generation and photic entrainment of circadian rhythms in mammals. Entrainment is mediated in part by a direct retino-hypothalamic tract (RHT), but other visual structures may also mediate photic effects on rhythms. A projection from the ventral nucleus of the lateral geniculate ($_{V}$LGN) has been identified anatomically as reaching the rat SCN. We conducted an electrophysiological study of single SCN and $_{V}$LGN cells in the rat to examine the functional organization of this pathway. We used antidromic stimulation techniques to identify the cells of origin of the $_{V}$LGN-SCN projection and to characterize their photic responsiveness. We also examined photic responses of SCN cells in rats which the $_{V}$LGN had been destroyed.

We recorded extracellular single-unit activity in the SCN and $_{V}$LGN of 38 urethane-anesthetized male Wistar rats; we also recorded SCN activity in 8 rats that had sustained complete bilateral $_{V}$LGN lesions. Photic stimuli consisted of diffuse white light of varying intensities presented to the eye contralateral to the $_{V}$LGN or SCN recording site. Cells projecting to the SCN were activated antidromically by applying constant current stimulation (0.1-0.5 mA, 0.1-0.2 msec) to the SCN while recording in the contralateral $_{V}$LGN. Recording and stimulation sites were verified histologically.

Visually responsive cells in the SCN responded tonically to changes in the level of retinal illumination. A large proportion of $_{V}$LGN cells in our sample (83/353) showed functional properties similar to those of SCN cells. Their receptive fields lacked an obvious antagonistic center-surround organization, and they either increased (67/83) or decreased (16/83) their firing rates tonically with increasing retinal illumination. A number of cells in the dorsal portion of the $_{V}$LGN could be activated antidromically by SCN stimulation with a latency of 3.0-6.5 msec. Their following of high-frequency stimulation and the results of collision tests indicated that these were genuine antidromic responses. Other $_{V}$LGN cells that projected to the SCN did not respond to either monocular or binocular retinal illumination. Destruction of the $_{V}$LGN did not alter the visual responsiveness of SCN cells.

These studies provide electrophysiological evidence for a $_{V}$LGN-SCN projection originating in cells near the dorsal border of the $_{V}$LGN. At least some of these units showed photic responses similar to those of SCN cells, but destruction of the $_{V}$LGN did not alter SCN photic responses. The functional significance of the existence of two similar visual inputs to the SCN remains to be established. (Supported in part by ZWO of The Netherlands.)

151.2 CIRCADIAN RHYTHM DISSOCIATION INDUCED BY PERIODIC FEEDING IN RATS WITH SUPRACHIASMATIC LESIONS. F. K. Stephan. Department of Psychology, Florida State University, Tallahassee, FL 32306.

Rats with lesions of the suprachiasmatic nucleus (SCN) were maintained in constant darkness and placed on restricted feeding schedules with 1h access to food twice per day. When both schedules had a 24h period and food access was spaced 12h apart, all rats with SCN lesions displayed anticipatory activity to both feedings. In the first experiment, the period of one schedule was then changed to 25h while the other continued with a period of 24h. None of the rats with SCN lesions were able to anticipate both schedules simultaneously. In a second experiment, 24h and 24.5h schedules were used. Five of six rats with SCN lesions anticipated both schedules for at least 9 days and 2 of these anticipated both schedules for 48 days. This forced dissociation of activity into two components with different periods is consistent with the hypothesis that entrainment of activity by restricted food access is mediated by more than one circadian pacemaker. These pacemakers are functionally independent of the SCN, a major pacemaker in the circadian system of rodents.

151.3 SUPRACHIASMATIC HYPOTHALAMIC NUCLEUS ORGANIZATION IN THE MACAQUE MONKEY: CYTOARCHITECTURE IN RELATION TO DISTRIBUTION OF RETINO-HYPOTHALAMIC AFFERENTS AND VASOPRESSIN NEURONS. R. Y. Moore and J. P. Card, Depts. of Neurology and Neurobiology, SUNY, Stony Brook, Stony Brook, NY 11794.

The suprachiasmatic nucleus (SCN) in the macaque monkey, as in other mammals, is the principal site of termination of retinal afferents to the hypothalamus (Hendrickson et al, 1972; Moore, 1973). The intent of the present study was to provide a detailed analysis of the distribution of the retinohypothalamic projection (RHT) to the SCN and relate this to the cytoarchitecture and the distribution of vasopressin (VP) neurons in the nucleus. In Nissl material the SCN appears rostrally as a thin nucleus above the optic chiasm comprised largely of small neurons. More caudally the SCN becomes oval and then round in appearance with the compact, small neuron population surrounded by a more loosely organized set of larger neurons. In material prepared by the autoradiographic tracing technique after injection of tritiated amino acid in one eye, dense labeling is found over the rostrocaudal extent of the SCN but concentrated in the ventral, parvocellular portion of the nucleus. Labeling is more dense in the rostral portion of the SCN than caudally and greater on the contralateral than the ipsilateral side. VP immunoreactivity is evident in neuronal perikarya and axons over the rostrocaudal extent of the SCN. The neurons are smaller and less densely stained than those in the supraoptic (SON) or paraventricular (PVA) nuclei and the axons are much finer than those arising from neurons of SON and PVA. The VP immunoreactive neurons of SCN are scattered through the nucleus and extend dorsally and laterally well beyond what would appear in Nissl material to be the confines of the nucleus. At mid-SCN, for example, VP perikarya and axons extend laterally to the border of SON and dorsally to the border of PVA. The center of the nucleus where the RHT terminates, however, is always free of elements exhibiting VP immunoreactivity.

These data indicate that the extent of the SCN in the macaque monkey is substantively greater than would be predicted from either cytoarchitecture or the area of termination of the RHT. As in the rat, VP neurons appear to be principally SCN interneurons which are segregated from the area of RHT termination. The similarities in organization of the SCN in rat and monkey suggest that the organization of the SCN in the generation of circadian rhythms is similar in these divergent mammalian species. Supported by NIH grant NS-16304.

151.4 AN ANALYSIS OF IMMUNOHISTOCHEMICALLY DISTINCT CELL AND FIBER SYSTEMS WITHIN THE SUPRACHIASMATIC NUCLEI OF THE GOLDEN HAMSTER BRAIN. J. P. Card and R. Y. Moore, Departments of Neurology and Neurobiology, SUNY, Stony Brook, Stony Brook, NY 11794.

The organization of the suprachiasmatic nuclei (SCN) of the golden hamster were investigated by cytoarchitectonic and immunohistochemical analysis. Examination of Nissl-stained coronal sections throughout the rostrocaudal extent of the optic chiasm (OC) reveal that the SCN consist of compact cellular masses situated over the caudal third of the OC on either side of the third ventricle. Each nucleus is approximately 650 μm in length and achieves its greatest height (600 μm) and width (300 μm) in the intermediate third of the rostrocaudal axis. With the exception of the rostral fourth of each nucleus, the SCN are fused along their ventromedial borders between the dorsal surface of the OC and the ventral limit of the third ventricle. Immunoperoxidase staining of tissue with antibodies generated against vasopressin (VP), vasoactive intestinal polypeptide (VIP; provided by J.Walsh), somatostatin (SS), avian pancreatic polypeptide (APP, provided by J. Kimmel), and 5-hydroxytryptamine (5-HT) reveal distinct and consistent subfields within each nucleus in all animals examined. VASOPRESSIN. Neurons displaying VP-like immunoreactivity are present throughout the rostrocaudal extent of the SCN and are relegated almost exclusively to the dorsal and medial aspects of each nucleus. A dense plexus of immunoreactive fibers is present in the dorsomedial aspect of the SCN as well as within a circumscribed area at the ventrolateral extent of the caudal third of the SCN/OC interface. VIP. Immunoreactive perikarya are absent from the rostral third of the SCN, but occur in large numbers throughout the remaining portions of the rostrocaudal axis. In this area, immunoreactive neurons are concentrated within the ventral third of the SCN throughout their mediolateral extent, including the ventral aspect of the area in which the two nuclei are fused. Immunoreactive fibers are found throughout the SCN, but are most dense in the dorsal half of each nucleus. SOMATOSTATIN. Immunoreactive perikarya are restricted to the dorsomedial aspect of the SCN with the major concentration occurring within the intermediate third of the rostrocaudal axis. Immunoreactive axons are present throughout the SCN with the densest concentration occurring within a vertical column filling the lateral third of each nucleus. APP. APP-like immunoreactivity is restricted to axons which form a dense plexus in the ventral and lateral aspects of the SCN. No immunoreactive axons are present in the dorsomedial portion of the nuclei. 5-HT. Axons exhibiting 5-HT-like immunoreactivity form a dense plexus within the ventromedial portion of the SCN. Scattered fibers also occur within the dorsal half of each nucleus. Supported by grant NS-16304.

151.5 GLUCOSE UTILIZATION OF THE SUPRACHIASMATIC NUCLEI IN THE DIURNAL SQUIRREL MONKEY. W.J. Schwartz, S. Eagan,* & M.C. Moore-Ede. Dept of Neurology, Massachusetts General Hospital & Dept of Physiology, Harvard Medical School, Boston, MA.

Accumulated evidence using a variety of techniques indicates that an endogenous circadian "clock" is located in the suprachiasmatic nuclei (SCN). A part of this evidence is from studies using the rate of SCN glucose utilization (measured by the ^{14}C-labeled deoxyglucose method) as a marker for the level of functional activity in the nuclei. In the rat, the SCN are metabolically active during the light period of a 12 hr : 12 hr light-dark cycle, and metabolically inactive during the dark (J comp Neurol 189:157). We now report our preliminary observations on SCN metabolic activity in the squirrel monkey (*Saimiri sciureus*). We initiated this study (a) to compare the pattern of SCN glucose utilization in this diurnal primate with that in the nocturnal rat, and (b) to determine whether visualizing the SCN in this larger mammal might allow resolution of metabolic heterogeneities corresponding to the localized distribution of SCN afferents and neuropeptides.

Adult male squirrel monkeys, weighing approx. 900 g, were housed in isolation chambers at approx. 26° C with food and water ad libitum and trained to sit quietly in metabolic chairs. Intravenous catheters were inserted at least 2 wks before the experiments. Unanesthetized animals were then injected either at mid-darkness or mid-light with 125 µCi/kg 2-deoxy-D-[1-^{14}C]glucose (sp. act. 60 Ci/mole). After 45 min, animals were sacrificed with pentobarbital; brains were frozen, 30 µ thick coronal sections cut, and autoradiographs prepared as described previously.

The SCN were active in monkeys injected during lights-on and relatively inactive during lights-off. Metabolic activity during the daytime was non-uniform both in its distribution and amount - the flattened anterior poles of the nuclei were more active than were the ovoid posterior poles. This autoradiographic image corresponded closely to the three-dimensional shape of the SCN described using anatomical reconstructions (Neurosci Lett 17:295).

Thus, the SCN of both a diurnal primate and a nocturnal rodent are metabolically active during the light portion of the day. Moreover, the observed metabolic heterogeneity within the squirrel monkey SCN may provide clues for future studies aimed at understanding SCN substructure and function.

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151.6

WITHDRAWN

151.7 GLUCOCORTICOIDS MEDIATE THE PHOTOPERIODIC CONTROL OF THE SIZE OF THE RECEPTIVE FIELD FOR GROOMING REFLEXES IN CATS WITH PONTILE LESIONS. R. F. Johnson* (SPON: W. Kaelber). Dept. of Psych., Univ. of Iowa, Iowa City, 52242.

Electrolytic lesions of the pons and adjacent tegmentum induce grooming reflexes in the cat. The grooming reflexes are elicited by tactile stimulation of the cat's skin, a stimulation which imitates the frequency, intensity, and excursion of the self-imposed stimulation produced when a cat normally grooms, and, the reflexes occur without the orientation to the skin seen in normally integrated grooming behavior. The physiological bases of the grooming reflexes involve a deficit of serotonin, confined to the superior colliculus, and a dysfunction of the seasonal rhythm in glucocorticoids.

The size of the receptive field for the grooming reflexes exhibits seasonal fluctuations that are controlled by photoperiod. Photoperiod also controls the seasonal rhythm of glucocorticoids in normal cats. An investigation was undertaken to determine if glucocorticoids mediate the photoperiodic effect on the size of the receptive field.

Four cats with lesion-induced grooming reflexes were bilaterally adrenalectomized and placed on a constant replacement therapy of hydrocortisone (3 mg/cat/day) and desoxycorticosterone pivalate (5 mg/cat/month). Six control cats exhibiting lesion-induced grooming reflexes were subjected to laparotomy. The size of the receptive field was allowed to stabilize while both groups were on a photoperiod of LD 17:7 and, subsequently, the groups were switched to LD 15:9, LD 10:14, and LD 8:16 for six weeks each. After the photoperiodic manipulations both groups (while stabilized at LD 8:16) received a single injection of L-5-hydroxytrytophan (5-HTP, 5 mg/kg).

The results indicated that the different photoperiods produced changes in the size of the receptive field of the control cats as predicted from previous longitudinal studies. The size of the receptive field of the adrenalectomized group remained constant during the photoperiodic manipulations. Both groups displayed a decrease in the size of the receptive field after the injection of 5-HTP.

It is concluded that glucocorticoids are a mediator of the photoperiodic effect on the size of the receptive field. Glucocorticoids are the critical factor removed by adrenalectomy as is indicated by the fact that neither mineralcorticoids nor adrenalin affect the size of the receptive field whereas hydrocortisone produces changes over a wide range of dosages. The efficacy of 5-HTP in the adrenalectomized cats indicates that photoperiod does not affect the size of the receptive field by effecting changes in serotonin and that the effect of serotonin does not require changes in the levels of glucocorticoids.

151.8 PHASE CHANGES OF EATING AND ACTIVITY CIRCADIAN RHYTHMS IN YOUNG AND OLD FEMALE RATS.
Z. M. Wenzel and P. K. Randall.* Andrus Gerontology Cntr., Univ. Southern California, Los Angeles, CA 90007

One aspect of temporal organization is the phase of various biological rhythms; disruption of these relations may impair homeostatic function. This study was designed to assess whether temporal disorganization, as indexed by changes in phases, occurs during aging in the rat.

Fifteen female, Fischer-344 rats (6-months-old (n=8) and 24-months-old (n=7)) were maintained ad libitum in a controlled environment under four sequential lighting conditions of 8 days each: LD:12:12, LL:24, DL:12:12 and DD:24, with a return to LD baseline for 8 days after each condition. All animals were videotaped continuously during the conditions, and several behaviors were scored. Spectral analysis was performed; the 24-hour period typically showed the highest spectral density. Phase was determined from the coefficients of the 24-hour period, and represents the hour of peak activity.

The phase of eating and activity changed across lighting conditions, as expected ($p<.001$). There was an age difference in the phase of eating ($p<.05$) and an age by condition interaction in the phases of both eating and activity (EAT: $p<.001$ ACT: $p<.05$). Young and old animals differed in eating and activity phases in the LD:12:12 condition (EAT: (mean hr±S.E.) young= 24.72±.32 old=23.75±29 ACT: young=26.46±.62 old=23.11± .97). Thus, old animals eat, and are active, earlier in the day than young in light-dark conditions. Alterations of phase resulting from different lighting conditions are dependent on both age and the behavior observed.

These data suggest that phases of the circadian components of the eating and activity rhythms change with age.

151.9 PHASE AND PERIOD OF FEMALE HAMSTER RUNNING RHYTHMS DURING THE ANNUAL REPRODUCTIVE CYCLE. L.P. Morin. Long Island Reseach Institute, Health Sciences Center, SUNY, Stony Brook, NY 11794

When given access to running wheels, female hamsters tend to initiate locomotor activity earlier on estrous cycle days when estradiol is available. The change in running onset time is probably caused by an estradiol-induced change in the circadian wheelrunning period. Because the phase angle difference (ϕ) in a light-dark cycle and the free running locomotor rhythm (τ) of female hamsters are sensitive to the effects of estradiol (Morin et al., Science 196:305, 1977), several experiments were designed to determine a) whether changes in ϕ of the wheelrunning rhythm occur following spontaneous recovery of estrous cycles during prolonged exposure to LD 6:18, b) whether τ changes in association with spontaneous estrous cycle resumption in constant darkness (DD) and c) whether changes in ϕ might be sufficiently great to promote photostimulation of animals still in LD 6:18. The experiments also permitted an assessment of the effect of wheel access on latency to LD 6:18 induced estrous cycle loss and resumption.

The results showed: 1) ϕ and τ shortened in all animals housed in prolonged LD 6:18 or DD; 2) abrupt changes in ϕ and τ were associated with spontaneous resumption of estrous cycles in LD 6:18 or DD; 3) after 25 wk in LD 6:18, there was wide variability in ϕ among the animals. The variability was greater than among animals housed in LD 6:18 for only 8 wk. With one exception, the greatest ϕ was not sufficient to permit photostimulation as predicted by an existing model (Elliott, J.A., Proc. 35:2330, 1976); 4) in general, ϕ in LD conditions predicted later τ; 5) loss of estrous cycles by animals housed in LD 6:18 occurred about 5.2 wk earlier if animals were housed without access to running wheels. Latency to estrous cycle resumption was roughly the same (24.4 ± 0.3 vs 23.5 ± 0.4 wk).

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151.10 ENTRAINMENT OF SPLIT CIRCADIAN RHYTHMS IN HAMSTERS. Z. Boulos and L.P. Morin. Long Island Research Institute, SUNY, Stony Brook, NY 11794.

Male hamsters that showed splitting of their circadian activity rhythms following long-term exposure to constant illumination (LL) were maintained under light-dark (LD) cycles with 2-h dark segments, and with periods of 24.00 h, 24.23 h or 24.72 h. In all cases, at least one of the split components entrained to the LD cycles. Under 24.00 h cycles, the onset of the entrained component preceded dark onset by 0.5-2 h, while under 24.23 h and 24.72 h cycles, the entrained component phase-led dark onset by about 3 h and 9 h, respectively. In some animals, the second split component continued to free-run (for up to 60 days) before finally merging with the entrained component, while in others, the second component also entrained to the LD cycles but maintained a constant phase angle of 8-13 h relative to dark onset. This latter result was obtained in cases where the period of the LD cycle was shorter than that of the split components as well as in cases where it was longer, implying that split components can be both phase-advanced and phase-delayed by 2 h of darkness.

Animals that showed stable entrainment of both split components were again allowed to free-run in LL for several weeks. The LD cycles were then reinstated, but instead of overlapping with the first component, as it did before, the dark segment was made to overlap with the second. The entrainment patterns which ensued were similar to the ones obtained initially, indicating that the two split components are affected by darkness in a qualitatively similar fashion. These results are evidence that the pacemaker system underlying circadian activity rhythms in hamsters is composed of two mutually coupled oscillators, or populations of oscillators, each with its own bidirectional phase response curve.

151.11 PINEALECTOMY SHORTENS THE PERIOD OF THE CIRCANNUAL BODY WEIGHT RHYTHM OF GOLDEN-MANTLED GROUND SQUIRRELS(SPERMOPHILUS LATERALIS). I. Zucker. Dept. of Psychology,University of California, Berkeley, CA. 94720.

Female golden-mantled ground squirrels were maintained from birth (June,1980) in a 14L:10D photoperiod at a temperature of 23±2°C. Between July 30 and August 5,1980 animals were pinealectomized (Pinx;N=11) or sham-pinealectomized (sham-Pinx;N=12). On September 19,1980 all animals were transferred to a 10L:14D photoperiod for the duration of the experiment. Body weight and reproductive condition were monitored at weekly intervals. Timing of the peak in body weight in 1980 did not differ between Pinx and sham-Pinx groups (Nov. 8±8 days vs Oct. 25±7 days); nor were trough weights achieved earlier in Pinx than in sham-Pinx squirrels (April 25,1981±10 days vs May 15±9 days, $p>.05$). However, the subsequent peak in body weight occurred earlier in Pinx than in sham-Pinx animals (Sept. 1, 1981±9 days vs Sept. 27±8 days, $p<.05$). The period of the circannual body weight cycle, measured peak to peak, was 296±12 days for Pinx animals and 337±5 days for sham-Pinx squirrels ($p<.008$). Absolute peak and trough weights did not differ between the groups. Thus, pinealectomy produces a substantial shortening of the period of the circannual body weight rhythm without affecting body weight regulation per se. These findings suggest that hormones of the pineal gland affect the period of circannual oscillations. The modification of the period of mammalian clocks by the endocrine system, previously established for circadian rhythms, has been extended to oscillations with a period of approximately one year. Endocrine influences on basic timing processes appear to be widespread.

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151.12 THE EFFECTS OF PROTEIN MALNUTRITION ON REM SLEEP IN RATS OF THREE AGE GROUPS. L. Cintra*, S. Diaz-Cintra*, W.Forbes and P.J.Morgane. Worcester Foundation for Expt. Biology, Shrewsbury, MA 01545

In order to extend our previous studies (Expt.Neurol. 57:440-450, 1977) on effects of protein malnutrition on REM sleep we analyzed the amount and circadian distribution of REM sleep in rats of 60,90 and 220 days of age using both a 12/12 light/dark cycle and 24 hour dark cycle. Previously we showed, using groups combining malnourished animals with dietary-reversal animals,that malnourished rats showed a significant increase in REM during the dark period. In the present studies, using animals maintained on an 8% or 25% protein diet we found no significant differences in amount of REM between normal and malnourished rats at 60 days of age but significant differences in total 24 hr REM at both 120 and 220 days of age. In calculating REM dark-light ratios between the age groups on both diets using a 12/12 light/dark cycle we found no differences between the 60, 90 and 220 day old normals vs malnourished. The total amount of REM per 24 hrs in the 25% animals showed a significant decrease between 60 and 120 days and from 120 and 220 days. The malnourished animals showed a significant decrease from 60 to 120 days followed by a significant increase from 120 to 220 days. In general,REM dark/light ratios in both normal and malnourished animals increase between 60 and 120 and 120 and 220 days and are not significantly different between the 25 and 8% animals in all age groups. On the 24 hr dark schedule there was no significant difference between 60 and 120 day groups in total 24 hr REM % but there was a significant decrease in amount of REM between 60 and 120 days and a non-significant decrease between 120 and 220 days. On the 24 hr dark cycle, comparing the 1st 12 hours with the 2nd 12 hr period, there was no significant differences between the 8 and 25% animals at 60 days of age but there were significant differences between groups at both 120 and 220 days of age. In comparing normals vs malnourished the groups do not show significant differences in dark/light ratios on the 12/12 cycle but in the 24 hr dark cycle we found a different REM distribution for the malnourished in that significantly more REM occurs in the dark at 120 and 220 days of age. A significant REM redistribution at 220 days on the 12/12 light/dark cycle, i.e., increase in REM in the light in malnourished rats compared to normals is one main finding as is the significant increase in REM in malnourished compared to normals on the 24 hr dark cycle at 220 days. Overall, it appears that whereas normal animals show decreased REM on both the 12/12 and 24 hr dark cycles, malnourished animals tend to increase total REM by 220 days on both cycles. The affects on REM in the 3 age groups may be related to morphological changes we have demonstrated in raphe and locus coeruleus and to the marked increases in biogenic amines we have shown in malnutrition. (Supported by Grant HD06364, NICHD)

151.13 ENTRAINMENT TO FOOD AND LIGHT SCHEDULES IN VMH LESIONED RATS. R. Mistlberger* (SPON: R. Rosenberg). Sleep Lab., Univ. of Chicago, Chicago, IL 60637

When access to food is restricted to a single mealtime every 24 hrs., rats and other mammals show increases in locomotor activity and changes in other physiological variables which anticipate the mealtime by 1-2 hrs. This anticipation appears to involve the entrainment of an endogenous circadian timing mechanism since it occurs only when the periodicity of food access is within the circadian range.

There is little information on the neural substrate of this mechanism. It appears to be anatomically separate from mechanisms controlling free running, light entrained rhythms since food entrained rhythms are not abolished by suprachiasmatic nucleus lesions as are most free running rhythms. There is one report that ventromedial hypothalamic (VMH) lesions prevent food entrainment of corticosterone and temperature rhythms of female rats in light-dark (LD) cycles (Krieger, D.T., Endocrinology 106:649, 1980). The present study addresses the following points: is the effect specific to females or the variables tested; is there recovery of function over time; and do VMH rats simply require longer exposure to food schedules before showing entrainment?

Tilt cage activity of 6 hyperphagic VMH male rats was continuously monitored in LD (lights on 9:00) and displayed on computer generated actograms. Four weeks following surgery food access was restricted to 11:00-13:00. Four rats showed consistent anticipatory activity within 4-12 days, whereas 1 showed weak and 1 showed no entrainment up to the 16th day. At twelve weeks following surgery food access was restricted to 11:00-13:00 in constant dim light. All the rats showed anticipatory activity, although the precise latency in some cases was obscured due to free running rhythms. The rat which did not entrain during LD food restriction showed consistent anticipatory activity by day 10, suggesting either a recovery of function or a difference based on ambient lighting. More rats will be tested to separate these possibilities.

Currently, 7 hyperphagic VMH females are being tested in LD. After 15 days of restricted feeding initiated 5 weeks following surgery 6 rats show no consistent anticipation. One rat showed anticipatory activity by day 10. As a group, the VMH females tend to be more obese and show worse entrainment than the males, suggesting a sex difference possibly relating degree of obesity with impairment of entrainment. Histological analysis will be done to assess equivalency of lesions.

Entrainment to LD cycles among the 13 VMH rats was variable. Some entrained to LD during ad-lib and restricted feeding, but not to mealtime, indicating that the effects of VMH lesions on LD entrainment can be independent of effects on food entrainment.

151.14 A CHEMICAL GATING THEORY OF CIRCADIAN RHYTHMS. G. A. Carpenter, Northeastern University, Boston, MA. S. Grossberg, Boston University, Boston, MA.

This work develops a model of circadian rhythms in which each term has a concrete physical interpretation. One key component of the pacemaker is a chemical which gates, or multiplies, signals by mass action, and which replenishes itself slowly. The pacemaker not only exhibits the properties which other models have, such as characteristic phase leads and lags and Aschoff's rule, but also, under certain circumstances, can exhibit period doubling (48-hour days) and spontaneous, long-term,periodic modulation of duration and intensity of activity (biorhythms). To account for experiments in which complex activity such as wheel-turning is the circadian rhythm being measured, the model is augmented to include the effects of fatigue and learned incentive. In this case the model accounts for such important properties as long-lasting aftereffects on period and duration of activity; and split rhythms for diurnal and nocturnal animals. The interactions within the model cause the chemical gating substance to oscillate with a circadian rhythm, as do various neural modulators and transmitter substances.

151.15 PINEAL MELATONIN INFLUENCES SEROTONIN METABOLISM IN THE AVIAN BRAIN. V.M. Cassone* and M. Menaker. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Indoleamine and catecholamine levels in the brainstem (pons and medulla) and diencephalon of 5 and 8 week old cockerels were determined by HPLC-EC. A daily rhythm of serotonin (5HT) content was found in the diencephalon; levels were higher at mid-day than at midnight ($p<0.01$). No day-night difference in 5HT could be determined in the brainstem nor could a day-night difference be demonstrated in either tissue for norepinephrine (NE), dopamine (D), homovanillic acid (HVA), 5-hydroxy-indoleacetic acid (5-HIAA) or n-acetyl serotonin (NAS).

Brains of cockerels pinealectomized at 2 weeks after hatching were also analyzed at 5 and 8 weeks as above. While pinealectomy had no effect on mid-day levels of 5HT in either the brainstem or diencephalon, midnight levels of diencephalic 5HT were elevated above levels found in intact controls ($p<0.01$) and above mid-day levels for both intact and pinealectomized cockerels ($p<0.05$).

Injections of melatonin (0.5 mg/kg I.P. in sesame seed oil) into pinealectomized cockerels two hours before sacrifice lowered midnight diencephalic 5HT content when compared to cockerels injected with oil at the same time ($p<0.01$). Brainstem 5HT content, however, was increased by melatonin injection ($p<0.05$). HPLC-EC analysis of these tissues for melatonin revealed an inverse correlation between diencephalic 5HT content and diencephalic melatonin content ($p<0.01$) and a direct relationship between brainstem 5HT content and brainstem melatonin content ($p<0.05$).

In addition, cockerels which had been injected with melatonin had elevated levels of diencephalic 5-HIAA ($p<0.01$). No effect of melatonin on brainstem 5-HIAA could be determined.

These results indicate that pineal melatonin may act by increasing 5HT turnover and/or release in the diencephalon and 5HT content in the brainstem. They suggest a link between the circadian secretion of pineal melatonin and the regulation of 5HT projections to the diencephalon from the raphe nuclei.

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151.16 PHOTIC REGULATION OF CYCLIC NUCELOTIDE LEVELS AND N-ACETYLTRANSFERASE ACTIVITY IN THE CULTURED AVIAN PINEAL. J.S. Takahashi and M. Zatz*. Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20205.

The avian pineal gland expresses a circadian oscillation of serotonin N-acetyltransferase activity and of melatonin release in vitro. In addition, light-dark cycles can entrain the rhythm of melatonin release in culture. Thus, the avian pineal contains a circadian oscillator and a photoreceptor, which regulate the synthesis of melatonin. Previous studies have suggested that cyclic nucleotides may be involved in the regulation of the enzyme, N-acetyltransferase. However, a clear relationship between cyclic nucleotide levels and enzyme activity has not been established.

We have measured cyclic AMP and cyclic GMP levels in cultured chick pineal glands and found that light exposure of the gland reduces the levels of both nucleotides. In pineals cultured for 6 hours in darkness, cAMP levels (57.5 ± 4.0 pmol/mg protein, $N = 54$, $\bar{x} \pm SE$) and cGMP levels (12.1 ± 0.9 pmol/mg protein, $N = 54$) were higher than those found in pineals exposed to 6 hours of light (cAMP: 14.9 ± 1.5, $N = 12$; cGMP: 5.28 ± 0.58, $N = 11$). Ten minutes of light exposure after 6 hours of darkness rapidly reduced cyclic nucleotide levels (cAMP: 19.7 ± 1.1, $N = 53$; cGMP: 4.43 ± 0.33, $N = 53$). The reduction of cAMP and cGMP levels by light preceded the reduction of N-acetyltransferase activity by light.

To determine which nucleotide, cAMP or cGMP, regulates N-acetyltransferase activity, the level of each nucleotide was increased by exposing pineals to selective stimulation by forskolin (an activator of adenylate cyclase) or to sodium nitroprusside (an activator of guanylate cyclase). Forskolin prevented the reduction in N-acetyltransferase activity by light; whereas sodium nitroprusside had no effect. Furthermore, addition of 0.25 mM 8-bromo cAMP prevented the light effect on enzyme activity; whereas, addition of 0.25 mM 8-bromo cGMP did not. Thus, the intracellular levels of both cAMP and cGMP are correlated with N-acetyltransferase activity. However, pharmacological experiments strongly suggest that cAMP, rather than cGMP, regulates the activity of this enzyme.

(J.S.T. is a Pharmacology Research Associate in the National Institute of General Medical Sciences.)

151.17 OPTIC EFFERENTS INFLUENCE PHOTIC ENTRAINMENT OF THE APLYSIA EYE CLOCK. Wesley P. Jordan and Marvin E. Lickey, Department of Psychology, University of Oregon, Eugene, OR 97403.

Each eye of Aplysia californica is a circadian clock. In constant darkness, a rhythm of compound action potentials (CAPs) can be recorded in vitro from the optic nerve of an isolated eye. Efferent fibers in the optic nerve contribute to the resetting of the phase of the CAP rhythm in vitro (Prichard & Lickey, 1981). This suggests that efferent activity also may influence the phase of the eye clock under entrained conditions in vivo. We tested this hypothesis in animals with one optic nerve cut before they were placed in 24-hr LD cycles of 1 to 16 hr of light per cycle.

The following table shows the median phases (relative to dawn) of the CAP rhythms from eyes attached to or isolated from the cerebral ganglion. There appeared to be two stable phases for both attached and isolated eyes in a 1-hr photoperiod. In both cases, one population of phases anticipated dawn by about 3 hr, while the other population of phases anticipated dawn by about 13 hr. This bistability disappeared in longer photoperiods. When the photoperiod was 2 or 6 hr, the phases of the rhythms were distributed unimodally, but the phases of attached and isolated eyes were different. While the CAP rhythm for attached eyes anticipated dawn by 1 to 3 hr in both LD 2:22 and LD 6:18, the phases of isolated eyes anticipated dawn by 12 hr in LD 2:22, but by only 5 hr in LD 6:18. The phase of the isolated eye rhythms appeared to follow dusk by 10 to 11 hr at these photoperiods. When the photoperiod was 9 hr or greater, the phases of both attached and isolated eyes were near dawn.

Photoperiod	Attached eye	Isolated eye
1	3 & 13	13 & 3
2	3	12
6	1	5
9	3	3
12	3	2
16	1	-1

Attachment to the cerebral ganglion in vivo affects the phase of the attached eye. If the phase of the rhythm from an isolated eye can be taken as the phase of the ocular pacemaker unaffected by the rest of the circadian system, then components of the system outside of the eye probably influence the phase of the ocular clock via efferent activity in the optic nerve. (Supported by NIH F32 NS06425 and NSF BNS 81-08168)

151.18 OPTIC NERVE PROJECTIONS TO CENTRAL GANGLIA OF APLYSIA. L. Olson* and J. Jacklet (SPON: R. Oesterreich). Dept. of Biol. SUNY Albany, Albany, N.Y. 12222

The optic nerve of Aplysia carries information from the circadian pacemaker located in the eye to the cerebral ganglion of the CNS. The clock driven synchronous activity of secondary neurons in the eye is high during the day and declines at night, coinciding with locomotor rhythms of the animal (Block, 1981). Studies have established that an intact optic nerve is necessary for normal expression of circadian locomotor rhythms (Lickey et al., 1976). We have examined the central projections of the optic nerve using horseradish peroxidase (HRP) backfilling of the optic nerve and ^{3}H-leucine labelling of cells in the eye with subsequent visualization of axon tracts by autoradiography.

HRP was introduced to the cut end of the optic nerve and processed for light or electron microscope analysis. These studies show that the optic nerve enters the dorsal surface of the ganglion parallel to the rhinophore nerve (RN) and continues within the sheath for a short distance before dipping into the ganglion. It then travels within the cell body layer, associated with a cluster of small opaque neurons between neuron clusters C and D of Jahan-Parwar and Fredman's (1976) classification, before entering the neuropil between cell clusters C and A. At this point the tract enlarges considerably, with a major bundle continuing posteriorly to leave the ganglion along the cerebral-pleural (C-P1) connective. In the neuropil area between clusters C and A a few fibers fan out laterally in both directions distinct from the C-P1 tract; these fibers often appear beaded. Efferent axons, from monopolar cell bodies located along the RN, enter the optic tract in this area, and branch or arborize in this same area of neuropil. Preliminary ultrastructural studies of HRP filled axons in the optic nerve proper show axon profiles which contain dense-core vesicles of a similar morphology to those described in the secondary cells of the eye (Luborsky-Moore and Jacklet, 1976).

Labelled fibers from neurons of eyes incubated in ^{3}H-leucine (10^{-6} M) confirm and extend HRP data. In addition to the large bundle of fibers exiting the ganglion along the ipsilateral C-P1 connective, these studies reveal another large tract which crosses the midline; a large group of these fibers leave along the contralateral C-P1 connective. Fibers leaving the ganglion may be terminating in the pleural ganglion, or may be contacting vascular spaces along the connective.

These studies have indicated an unexpectedly extensive projection of optic fibers within the Aplysia CNS, consistent with the idea that clock information may be widely distributed in the CNS to pattern daily behavior. Continuing studies are examining ultrastructural details of optic nerve projections.

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151.19 EFFECT OF EXTERNAL LEVEL OF CALCIUM ON THE CIRCADIAN ACTIVITY OF THE CRAYFISH. Beatriz Fuentes-Pardo, Leticia Verdugo-Díaz† and Virginia Inclán-Rubio†. Depto. de Fisiología, División de Investigación. Facultad de Medicina. U.N.A.M. México 20, D.F. México.

Calcium has been recognized as an important element in many infradians or high frequency cellular oscillators suggesting that periodical activity is a common property of most biological systems. It has been also suggested that a population of high frequency interacting oscillators can give rise to circadian oscillations, i.e., it is possible that some calcium- dependent oscillators when coupled, can generate a twenty-four hours rhythm We have approached this problem by investigating wheather the electrical response to light (ERG) recorded from the isolated eyestalk of crayfish during four or more days, is modified by changes in the external level of calcium. The excised eyestalk were immersed in normal, calcium-free or calcium-rich saline solution and both, ERG and the position of the shielding pigments were obtained. The ERG amplitude showed a circadian rhythm with periods about 20 or 26 hours in calcium-free or calcium-rich saline solution respectively. Besides the circadian changes the isolated eyestalk showed superimposed high-frequency oscillations (two hours period) that were also modified by calcium external level. The shielding pigments remained in the light adaptation position and there were not any noticeable circadian oscillation in either calcium-free or calcium-rich solution. Since circadian changes were not observed in the shielding pigment position, the ERG fluctuation is not attibutable to changes of the input, i. e. the external level of calcium is not affecting the position of pigmentary effectors but is acting on the receptor level. The fact that the eyestalks in isolation exhibit changes in its ERG circadian rhythm depending of the calcium level, strongly supports the idea that calcium plays an important role on some oscillators involved in the generation of the ERG circadian rhythm, in particular those responsible of the high frequency oscillation.

151.20 A PROTEIN SYNTHESIS INHIBITOR BLOCKS THE EFFECT OF SEROTONIN AND 8-BENZYLTHIO-cAMP ON THE APLYSIA EYE CIRCADIAN RHYTHM. A. Eskin, Dept. of Biology, Univ. of Houston, Houston, TX 77004.

Serotonin (5-HT) phase shifts a circadian rhythm in the Aplysia eye. The identification of cellular mechanisms involved in phase shifting by 5-HT should help in determining the molecular components of the circadian oscillator because 5-HT must perturb some part of the oscillator to phase shift the rhythm. Our previous work established that 5-HT phase shifts by activating adenylate cyclase and increasing cAMP. We recently began experiments to determine if protein synthesis is required for phase shifting by 5-HT because inhibitors of protein synthesis phase shift the rhythm (Rothman and Strumwasser, 1976, Jacklet, 1977).

We compared phase shifts produced by various 6 hr treatments in the presence and absence of the protein synthesis inhibitor anisomycin (aniso). Aniso rapidly and reversibly inhibits protein synthesis by over 90% in the abdominal ganglion and eye and has no effect on many neurophysiological parameters (Schwartz et al, 1971, Jacklet, 1980). At phase CT06-12 aniso (10^{-6}M) by itself does not phase shift (-0.1±0.7 hr, N=5, 95% C.I) and 5-HT (10^{-5}M) produces an advance phase shift (+2.5±1.5, N=7). The phase shift normally produced by 5-HT was significantly inhibited when eyes were treated with 5-HT plus aniso (+0.6±1.4, N=5). To investigate the specificity of the inhibitory action of aniso, we determined whether other effects of 5-HT in the eye, which also may be mediated by cAMP, were blocked by aniso. 5-HT, during the second hour of treatment, inhibits by 90% spontaneous neural activity from the eye. A similar amount of inhibition (99%) was produced by 5-HT in the presence of aniso. 5-HT produces a large and long lasting increase in the photosensitivity of the eye as measured by ERGs. Aniso also did not interfere with the effect of 5-HT on the ERG. These data suggest that aniso blocks the phase shifting effect of 5-HT at some point beyond cAMP. Examining this idea, we found that the phase shift normally produced by 10^{-3}M 8-benzylthio-cAMP (+3.1±0.8, N=10) was absent when eyes were treated with 8-BT-cAMP plus aniso (+0.9±0.5, N=8). All together these results suggest that 5-HT phase shifts the rhythm by increasing cAMP which then leads to an increase in protein synthesis. Thus, a protein synthetic event appears to be a cellular mechanism involved in phase shifting by 5-HT. It is possible that this synthetic event is part of the clock machinery of the cell. 5-HT may now be used as a probe to identify the synthetic events that are important for circadian timing. Our results are particularly interesting with respect to information processing because very few effects of transmitter substances are blocked by protein synthesis inhibitors.

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152.1 *IN VITRO* β-ADRENERGIC STIMULATION OF PINEAL MELATONIN PRODUCTION AND N-ACETYLTRANSFERASE ACTIVITY IN THE RAT. C. M. Craft and R. J. Reiter. Dept. of Anatomy, The Univ. of Texas Health Sci. Ctr., San Antonio, TX 78284.

Norepinephrine (NE), released from post ganglionic sympathetic fibers, stimulates melatonin production and secretion by augmenting the activity of N-acetyltransferase (NAT). *In vitro* studies of the rat pineal gland stimulated with β-adrenergic agonists are well established. The usual parameter measured in these studies is NAT activity which is purported to be the rate-limiting enzyme for melatonin synthesis. The purpose of this study was to examine the responsiveness and time course of NE stimulated cultured glands by measuring melatonin content (ng/gl/h) by radioimmunoassay (RIA) and comparing it to NAT activity (pm/gl/h) in the same pineals. Male Sprague-Dawley rats were maintained in a photoperiod of daily 14:10 LD cycle (lights on at 0600 h) three weeks prior to culturing. Individual glands were cultured in BGJ_b medium for at least 24 h before NE stimulation. Initially, melatonin concentration in the medium was measured by RIA to determine the viability of the culture over a four-day period without NE. The melatonin level on day 2 (37 ng ± 3) or day 3 (39 ng ± 3.4) was significantly higher ($p<.001$) than on day 1 (22 ng ± 2) or day 4 (20 ng ± 1.8). NAT activity at the end of the 4 day period was low. In another experiment, the dose response to NE (10^{-5}M to 10^{-8}M) was analyzed after 48 h in culture. Melatonin levels (1 ng ± .1 to 6 ng ± .8) in the glands and NAT activity (2.3 pm ± 1.2 to 47 pm ± 8) were both dose dependent and significantly greater than controls when stimulated for 3 h with 10^{-5}M and 10^{-6}M. The melatonin levels in the medium (2.3 ng ± .05 to 3 ng ± .6) were not significantly different at any concentration. In the last experiment, the time course study used 10^{-6}M NE after 24 h initial incubation and the glands and media were analyzed at .5, 3.5, 6, and 24 h. Both melatonin content (glands: 2 ng ± .6 to 10 ng ± 2.8; media: 7 ng ± 3.8 to 20 ng ± 2.5) and NAT activity (48 pm ± 12 to 315 pm ± 21) at 3.5 and 6 h were significantly different than the 6 h control. The melatonin content shifted from the glands to the medium with the greatest amount in the medium after 24 h, while NAT activity was low at this time point. These data indicate a much greater melatonin content as measured by RIA in the glands and media as compared to earlier methods which measured [^{3}H]-melatonin by thin layer chromatography. The NAT activity during 24 h in culture is similar to reported values. Therefore, we suggest that the NE stimulation of pineal glands *in vitro* shows a dose response and time course response which can be quantitated in the glands and media by measuring radioimmunoassable melatonin and NAT activity. (Supported by NSF Grant No. PCM 8003441.)

152.2 ALTERATIONS IN PINEAL N-ACETYL-TRANSFERASE ACTIVITY AND MELATONIN CONTENT IN MALE SYRIAN HAMSTERS RENDERED DIABETIC WITH ALLOXAN. Thomas H. Champney, George C. Brainard and Russel J. Reiter. Dept. Anatomy, The Univ. of Texas Health Sci. Ctr., San Antonio TX 78284.

Recently, insulin was shown to be the most effective inactivator of pineal N-acetyl-transferase (NAT) activity in an *in vitro* broken cell preparation (Namboodiri, M. A. A., J. T. Favilla and D. C. Klein, 1981, Science, 213: 571). The purpose of this study was to test the *in vivo* effects of insulin on pineal melatonin content and NAT activity. Syrian hamsters, rendered diabetic by an intravenous injection of alloxan monohydrate (60 mg/kg), were maintained under long photoperiods (14:10 LD) with lights on at 0600. Two separate experiments were conducted. For the first experiment, control and diabetic animals were killed at 2000, 0200 and 0400h three days after alloxan injection. In the second study, animals were killed at 1600, 2400, 0400 and 0600h three days after injection. Pineals and trunk blood samples were collected. Blood glucose levels were determined by glucose test strips (bG Chemstrip, Bio-Dynamics). Control hamsters had blood glucose values of approximately 40 mg/dl. Hamsters with blood glucose values over 180 mg/dl were considered diabetic. Pineal NAT activity was measured by a radioenzymatic assay for the first experiment only, while pineal melatonin levels were measured by radioimmunoassay for both experiments. NAT levels were significantly ($p<0.05$) increased by alloxan diabetes at 2000h, but were unaffected at 0200 and 0400h. However, melatonin levels were higher than those in corresponding control hamsters at 1600h ($p<0.02$), but lower at 2000 ($p<0.02$), 0400 ($p<0.01$) and 0600 h ($p<0.01$). These results indicate that experimentally-induced diabetes, presumably through depressed levels of insulin or increased titers of blood glucose, can alter the melatonin content of the hamster pineal gland. The mediation of reproductive and neuroendocrine processes by the pineal is well documented (Reiter, R. J., 1981, Am. J. Anat., 162: 287-313). Therefore, the observed alterations in pineal melatonin content may have subsequently affected other endogenous rhythms. Further experiments are planned to examine the physiological consequences associated with these effects. (Supported by NSF Grant No. PCM 8003441.)

152.3 THE INFLUENCE OF LOW IRRADIANCES OF BLUE AND GREEN LIGHT ON PINEAL MELATONIN CONTENT IN THE SYRIAN HAMSTER. G. C. Brainard, B. A. Richardson*, T. S. King, and R. J. Reiter. Dept. Anat., Univ. Texas Health Science Center, San Antonio, TX 78284.

Cool white fluorescent light irradiances of 0.186 μW/cm² or above suppress nocturnal pineal melatonin content in the Syrian hamster, whereas irradiances of 0.019 μW/cm² or below do not suppress pineal melatonin (Brainard et al., Brain Res. 233, 75-81, 1982). Using red, yellow, green, blue and near-ultraviolet fluorescent light sources at irradiances of 0.928 and 0.20 μW/cm², it has been demonstrated that blue and green fluorescent light produce the greatest depression of nocturnal melatonin in the hamster (Brainard and Reiter, Endocrinology, Suppl., 1982, in press). The purpose of the following study was to determine which color of light, blue or green, is most efficient for suppressing nocturnal pineal melatonin content. Three sets of 21 adult male Syrian hamsters each were adapted to a light:dark cycle of 10:14 (lights on 0700 hours). In each set, 7 animals each were exposed to either green light, blue light or darkness during the nocturnal melatonin peak (0200-0500 hours). The irradiances used for each set were 0.186, 0.074 or 0.019 μW/cm². After twenty minutes of exposure, pineals were removed from animals and later assayed for pineal melatonin content by radioimmunoassay. Data were analyzed initially by one-way ANOVA and significance established by Newman-Keuls test. Animals exposed to 0.186 μW/cm² of either blue or green fluorescent light for twenty minutes had significantly ($P<0.001$ and $P<0.01$, respectively) suppressed pineal melatonin contents compared to those of unexposed animals. Animals exposed to 0.074 μW/cm² of blue light had significantly suppressed pineal melatonin contents ($P<0.01$) compared to those of unexposed animals. In contrast, animals exposed to 0.074 μW/cm² of green light had pineal melatonin contents which were not significantly different from those of unexposed animals. Finally, animals exposed to 0.019 μW/cm² of either blue or green light had pineal melatonin contents which were not significantly different from those of unexposed animals. At each irradiance tested, blue light caused a 20% to 25% greater depression of pineal melatonin compared to that induced by green light. These data demonstrate that the blue wavelengths of light are the most efficient wavelengths of visible light for suppressing nocturnal pineal melatonin content in Syrian hamsters. These findings indicate that photic control of the pineal gland may be at least partially chromatic. (Supported by NSF Grant No. PCM 8003441.)

152.4 EFFECTS OF PHOTOPERIOD ON N-ACETYLINDOLEALKLAMINES IN SERUM, PINEAL, RETINA, & HYPOTHALAMUS OF THE GOLDEN HAMSTER. L.J. Grota and G.M. Brown. Dept. of Psychitary, University of Rochester, Rochester, NY 14642 and Dept. of Neuroscience, McMaster University, Hamilton Ontario

Male golden hamsters were housed in light:dark (LD) cycles of 14:10, LD 2:22, or LD 22:2 for 8 weeks when they were killed at 3 hour intervals beginning 1 hour after the onset of darkness. Immunoreactive melatonin was determined in pineal, retina, and hypothalamus by immunohistochemical methods on frozen 10 μ sections. N-acetylserotonin and melatonin in serum were determined by specific radioimmunoassay. Testes were weighed at the time of sampling. Testes weights did not differ among LD 14:10 and 22:2 animals (3.34 ± 0.04 gm, N=45 and 3.32 ± 0.01 gm, N=80) but these were heavier than LD 2:22 animals (1.11 ± 0.09 gm, N=79). Within the LD 2:22, testes weights showed a 24-hour rhythm with the heavier weights at 13:00 and 16:00 hours relative to the other 6 points measured (Lights on at 0:00) but these values were below those from LD 14:10 and LD 22:2. No 24-hour or light:dark rhythm of immunoreactive melatonin was observed in hypothalamus or in retina of animals from any LD cycle. In contrast to these data, immunoreactive melatonin in sections from pineal showed a 24-hour rhythm with a crest 6-8 hours after dark onset in LD 14:10 and LD 22:2 but this rhythm was not observed in LD 2:22. N-acetylserotonin levels in serum did not display a 24-hour rhythm in LD 14:10 and LD 22:2 but there was a rhythm in serum N-acetylserotonin in LD 2:22 with a crest just after the onset of darkness ($F=3.26$, $df=7/66$, $p < .01$). Melatonin levels are being determined at this time.

In a second study, male golden hamsters housed in LD 12:12 were placed into LD 2:22 (lights on at 0:00) and killed 0, 0.5, 1, 2, 4, or 8 weeks later. Gonadal weight and serum were obtained. Testicular weights are maintained from 2.5 and 3.5 gm for 4 weeks and atrophy to 0.875 gm by 8 weeks. Serum N-acetylsertonin levels were higher at 18:00 hours (dark) than at 6:00 hours (light) when animals were housed on LD 12:12). By 4 weeks in LD 2:22, N-acetylserotonin levels were higher at 6:00 than at 18:00 hours. Melatonin levels were significantly elevated .5 (111.5 ± 21 pg/ml, N=12) and 4 (636 ± 115 pg/ml, N=15) weeks after the onset of LD 2:22 relative to animals on LD 12:12 (25 ± 22 pg/ml, N=12). These data indicate that N-acetylserotonin and melatonin levels in hamster serum are altered by short photoperiod and that these changes preceed atrophy of the testes.

152.5 SEASONAL VARIATION IN RAT PINEAL VASOTOCIN. M.M. Prechel*, T.K. Audhya* and D.H. Schlesinger* (SPON: G.L. Humphrey). Department of Biochemistry and Biophysics, Loyola University Medical Center, Maywood, IL 60153.

Arginine vasotocin (AVT) has been proposed as a principle pineal hormone, yet there is little agreement in the literature concerning endogenous levels of AVT, and physiological variations in these levels. Major inconsistencies in the amount of AVT-immunoactivity extractable from rat pineal glands in this laboratory over a period of years, led to the study of pineal AVT-activity as a function of season.

Rats (King, Oregon, WI) were housed in L:D 14:10 with water and Purina rat chow ad libitum. Groups of 24 or 40 males and females, 28-30 days old, average 80-85 grams, were sacrificed each week between 8:15-9:30 a.m. (Central Standard Time) from July 1980 through September 1981. Pineal glands were removed, pooled, homogenized in 0.1 N acetic acid with 10^{-6}M pepstatin (an acid protease inhibitor), and then heat denatured to precipitate large proteins. Supernatants were studied by AVT radioimmunoassay (Fernstrom, et al., Endocrinology 106:243, 1980).

For most of the year pineal AVT-immunoactivity ranged between 1.8 and 7.7 pg/gland; the average value from Sept. through July was 4.1 ± 0.3 pg/gland (mean ± S.E.M.: N=48). However, both years in early August pineal AVT-activity increased several hundred fold. A value of 1720 pg/gland was measured on 8/16/80, and 1170 pg/gland on 8/10/81.

Thus we conclude that the basal level of rat pineal AVT-activity is extremely low (4.1 pg/gland), but that the activity increases dramatically at a precise time each year. This seasonal fluctuation provides an explanation for the inconsistency in previously reported rat pineal AVT-activity values.

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152.6 THE EFFECT OF OLFACTORY BULBECTOMY ON PINEAL INDOLES IN THE RAT. George M. Anderson*, Augustus R. Lumia*+, Bennett A. Shaywitz, J. Gerald Young and Donald J. Cohen*. Labs. Develop. Psychobiol. & Neurochem., Yale Univ. Sch. of Med., New Haven, CT 06510 & +Dept. Psychology, Skidmore College, Sartoga Springs, NY 12866.

Olfactory bulb removal results in profound changes in the sexual behavior and development of male and female rats. Neuroanatomical and neurochemical studies suggest that the effects are mediated through the hypothalamus. However, clear changes in hypothalamic neurotransmitter levels or steroid receptors have not been previously observed. The known effects of pinealectomy in causing precocious sexual development, the influence of the estrous cycle on rodent pineal indoles, and the importance of the suprachiasmatic nucleus in regulation of diurnal rhythms in the pineal have prompted us to examine the effect of bulbectomy on the pineal. Sprague Dawley rats were bulbectomized or sham-operated at 5 days of age then raised in mixed litters. At approximately 60 days of age the animals were sacrificed during the dark period (12hr light/dark cycle) and the brain and pineal removed. The brain was dissected into hypothalamus, striatum, pons, and cortex. Pineal indoles and brain neurotransmitters and metabolites were measured by HPLC with fluorometric and/or amperometric detection. A significant (p=.05) lowering of pineal melatonin (MEL) was seen in the bulbectomized animals (MEL 1.43 ± .25, N=22, 14M/8F) compared to the sham animals (MEL 1.96 ± .26, N=30, 16M/14F). No sex differences were seen in the MEL levels of bulbectomized animals (means 1.41 and 1.45) and no significant sex difference was seen in the sham animals (male 2.16 ± .28, female 1.74 ± .24). A complimentary change was seen in the serotonin (5-HT) levels of bulbectomized animals: a significant (p=.04) increase being observed in pineal 5-HT of bulbectomized animals (5-HT 116 ± 12, N=17) compared to sham animals (5-HT 87.0 ± 12, N=25). We will present results of the analyses of additional pineal indoles (tryptophan, 5-hydroxytryptophol, and 5-hydroxyindoleacetic acid 5-HIAA), and brain neurotransmitters (norepinephrine, dopamine, and 5-HT) and metabolites (homovanillic acid) and 5-HIAA). The changes seen and the implications of a demonstrated neurochemical connection between the olfactory bulb and the pineal will be discussed.

152.7 PINEAL-INDUCED ALTERATIONS IN PROLACTIN SYNTHESIS AND SECRETION IN BLINDED FEMALE HAMSTERS, K.M. Orstead* and B. Benson. Department of Anatomy, University of Arizona, Tucson, AZ 85724.

Hamsters subjected to optic enucleation or short photoperiods show marked depressions in pituitary and blood prolactin (PRL) levels. These effects are either reversed or prevented by removal of the pineal gland. This study was designed to determine whether pinealectomy would prevent the reduced in vitro synthesis and release of PRL by pituitaries from blinded female hamsters.

Adult, female golden hamsters were either blinded, blinded and pinealectomized (blind/pinx) or blinded and sham-pinealectomized (blind/sham). Controls were either left intact, pinealectomized (pinx) or sham-pinealectomized (sham). After surgeries, all animals were maintained in a long (L:D=14:10) photoperiod for 12 weeks. Upon sacrifice, homologous pairs of hemipituitaries were incubated for two hr at 37°C in 1.0 ml of oxygenated Kreb's Ringer bicarbonate buffer containing 100 mg% glucose plus 20 μCi ^{3}H-leucine. Serum, pituitary and media PRL-like immunoreactivities (PRLa) were estimated with the use of NIH anti-sera to rat PRL, rPRL-I-5 for iodination and either a standard hamster serum or pituitary pool. Presumptive, newly-synthesized ^{3}H-PRL in pituitary and media samples was separated by polyacrylamide disc gel electrophoresis and the incorporation of radiolabelled precursor quantified by liquid scintillation spectrophotometry.

Serum levels of PRLa in blind and blind/sham groups were significantly depressed when compared to controls. Pinealectomy of blinded hamsters completely prevented the depressions in serum PRLa levels. Both the content and concentration of PRLa in pituitaries of blind and blind/sham groups were significantly reduced when compared to controls. Also, pinealectomy totally prevented the reductions in pituitary PRLa levels of blinded animals. In addition, both the PRLa content and concentration in incubation media from blind and blind/sham groups were significantly reduced when compared to controls. Pinealectomy of blinded hamsters prevented these depressions, completely restoring the ability of their pituitaries to release PRLa into the incubation media. Furthermore, the total amounts of ^{3}H-PRL synthesized by glands from blind and blind/sham groups were significantly less than those of controls. However, pinealectomy of blinded animals only partially prevented the reductions in ^{3}H-PRL synthesis in vitro.

From these results, we conclude that pituitaries from blinded female hamsters show a significantly diminished ability to synthesize and release PRL in vitro. This phenomenon appears to be pineal-dependent, since pinealectomy completely prevents decreased release, and partially prevents depressed synthesis of PRL in vitro. Supported by NIH grant HD-08795.

152.8 5-METHOXYTRYPTAMINE: EFFECTS ON THE NEUROENDOCRINE-REPRODUCTIVE AXIS OF THE FEMALE SYRIAN HAMSTER. B. A. Richardson*, M. K. Vaughan, T. S. King, L. J. Petterborg*, and R. J. Reiter. Dept. of Anatomy, The Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

In the Syrian hamster, the pineal transduces ambient photic information into a chemical signal that determines the level of reproductive activity. Although the hormone(s) involved in this response remain an enigma, the pineal methoxyindole, melatonin (Mel), is the leading candidate. However, recent data on male hamsters (Pévet et al., 1981, J. Neural Transm. 51: 303) suggest that another pineal 5-methoxyindole, 5-methoxytryptamine (5-MT), may play an important role in pineal-mediated gonadal collapse in the Syrian hamster. Therefore, to determine the influence of 5-MT on the reproductive physiology of female Syrian hamsters, adult animals were maintained in a 14:10 (lights on at 0600h) light:dark cycle and divided into the following groups: 1) injected daily (1700h) with vehicle and received weekly implants of 25 mg beeswax pellets; 2) injected daily (1700h) with 25 μg Mel; 3) injected daily with Mel and received weekly implants of Mel pellets (1mg Mel in 24 mg beeswax); 4) injected daily with Mel and received weekly implants of 5-MT (1 mg 5-MT in 24 mg beeswax); 5) injected daily (1700h) with 50 μg 5-MT; 6) injected daily with 5-MT and received weekly implants of Mel; and 7) injected daily with 5-MT and received weekly implants of 5-MT. All female hamsters receiving 25 μg Mel injections for 9 weeks became acyclic. These animals also demonstrated a significant ($p<0.001$) decrease in uterine weight. Mel injections also resulted in a marked suppression of both pituitary ($p<0.001$) and plasma ($p<0.001$) prolactin (PRL) whereas the gonadotrophins (LH and FSH) were unaffected. The influence of afternoon injections of Mel on estrous cyclicity and uterine weight, but not pituitary and plasma PRL, were negated by either Mel or 5-MT implants. Nine weeks of daily 5-MT injections resulted in 78% of these animals being acyclic and significant decreases in uterine ($p<0.001$) weight and pituitary ($p<0.001$) and plasma ($p<0.001$) PRL. Like Mel injections, the effects on cyclicity and uterine weight were completely blocked by either Mel or 5-MT implants. These results suggest that 5-MT, like Mel, can have an inhibitory influence upon the reproductive physiology of female Syrian hamsters. The ability of both Mel and 5-MT pellets to prevent the actions of either indole when injected implies that the site(s) of action is the same for Mel and 5-MT. However, since the inhibitory influence of 5-MT in the present paradigm was less than Mel at twice the dose, it is felt that 5-MT is a less physiologically active compound than Mel. (Supported by NIH Postdoctoral Fellowship 1 F32ND 05900 to BAR, HD 07139 to TSK and NSF Grant No. PCM 8003441 to RJR.)

152.9 COUNTER-ANTIGONADOTROPIC EFFECT OF MELATONIN ADMINISTERED VIA THE DRINKING WATER. Finley P. Gibbs* and Jerry Vriend* (SPON: David E. Scott) Departments of Anatomy University of Missouri, Columbia, MO 65212, and University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3

Our hypothesis was that since hamsters drink 80-90% of their water at night and since plasma melatonin levels are high at night that we could augment the natural fluctuation of plasma melatonin by putting it in the drinking water. Further, since injections of melatonin are most effective in producing gonadal atrophy when given late in the photoperiod, melatonin given via the drinking water could be equally effective. Preliminary experiments using RIA indicated that doses in the range of 2 to 5 ug/ml in the drinking water would produce a circadian rhythm of plasma melatonin approximately in the physiologic range (10 to 100 pg/ml). Melatonin was dissolved in ethanol and then further diluted in tap water to concentrations ranging from 0.059 ug/ml to 320 ug/ml. The solutions were prepared twice a week, and the drinking bottles were covered with aluminum foil to protect the melatonin from damage by light.

Two protocols were used, one to demonstrate the antigonadotropic effect of melatonin and the other to elicit the counter-antigonadotropic effect. In the antigonadotropic protocol 100 g intact male hamsters were housed in a 14:10 LD cycle four or five per cage for ten weeks. Contrary to our hypothesis there was no effect of melatonin in the drinking water on testis weight (dose range: 0.059-50 ug/ml). The counter-antigonadotropic protocol involved the use of 100 g male hamsters which were blinded and housed under similar conditions. They received melatonin in concentrations ranging from 1.8-320 ug/ml. At ten weeks the blinded hamsters receiving no melatonin had the expected drop in testis weight to 0.56 g. In the range of 2.4 to 10 ug/ml there was a highly variable response, but generally higher doses resulted in higher testis weights. In the range of 20-320 ug/ml the blind hamsters had mostly normal appearing large testes (mean = 3.2 g).

In conclusion: 1) We produced the well known counter-antigonadotropic effect of melatonin by administering it via the drinking water. 2) We could not produce the anti-gonadotropic effect. (Supported in part by funds from the University of Missouri's Biomedical Research Support Award and the Manitoba Health Research Council).

152.10 CORTICOSTERONE AND N-ACETYLSEROTONIN (NAS) RESPONSES TO STRESS: EFFECT OF HOUSING CONDITIONS AND TYPE OF ENVIRONMENTAL STIMULATION. Jo Seggie, G.M. Brown, L.Campbell* and L. Grota, Departments of Neurosciences & Psychiatry, McMaster University, Hamilton, Ontario, L8N 3Z5.

Preliminary observations in our lab suggested that NAS may be stress responsive. NAS is a hormone secreted by the pineal gland whose synthesis in the pineal is regulated by a B-adrenergic sympathetic input which originates in the superior cervical ganglion. Since stress elevates circulating norepinephrine and epinephrine and the pineal lacks a blood brain barrier we investigated the possibility that NAS is responsive to various types of environmental stimuli under different housing conditions. Separate groups of animals were either sacrificed in the undisturbed state or exposed for three minutes to a variety of stimuli (novel environment, cold water, noise and ether vapour) and then decapitated for collection of blood at 0, 5, 15, 30 or 60 minutes after the end of stimulation. Corticosterone was measured in the same samples as an indication that animals responded to these stimuli. These experiments were conducted at the beginning of the light phase of a 12 hr. light/dark cycle. All subjects had ad lib food and water but half of each group were housed singly, half were housed in groups of four. Corticosterone levels, measured by competitive protein binding evidenced an increase to all stimuli. Cold water elicited the largest response while the other stimuli had equivalent responses. Housing condition did not effect the response. NAS levels measured by specific radioimmunoassay were found to interact with time and type of stimulation. Housing conditions had no effect on NAS levels. Cold water, novel environment and noise did not affect NAS levels. In contrast to these data three minutes of ether exposure resulted in an immediate drop in plasma NAS levels.

These data indicate that NAS does not rise in response to stimuli which produce a corticosterone rise and presumably a catecholamine rise. In fact ether produced a rapid drop.

Supported by funds from the Ontario Mental Health Foundation (OMHF) and Medical Research Council of Canada. Jo Seggie and G.M. Brown are Research Associates of the O.M.H.F.

153.1 CHRONIC SYMPATHETIC DENERVATION ALTERS PHOSPHATIDYLINOSITOL RESPONSE IN THE PAROTID GLAND, M. R. Hanley *, M. D. Dibner and C. P. Downes* (SPON: S. H. H. Chan). *MRC Neurochemical Pharmacology Unit, Cambridge University, Cambridge CB2 2QH England.

The parotid gland of the rat responds to agents such as substance P, carbachol and phenylephrine with a phosphatidylinositol (PI) response. This can be measured by ^{3}H-inositol incorporation, ^{32}P incorporation into PI, or polyphosphoinositide formation. Adult Sprague-Dawley rats underwent unilateral superior cervical ganglionectomy or sham operation to determine whether adrenergic denervation affected the PI response. After 10-14 days, the incorporation of ^{3}H-inositol into PI following substance P, carbachol or phenylephrine stimulation was greatly reduced in the operated side as compared with that seen in innervated contralateral parotids or in tissue from sham-operated control rats. Further studies indicated that sympathetic denervation led to a decrease incorporation of ^{32}P into PI. In contrast, phosphatidic acid synthesis and inositol-1-phosphate formation were normal. Moreover, it appears that the rapid decrease in triphosphatidylinositol stimulated by substance P may be attenuated in denervated salivary glands. Thus, sympathetic denervation of the rat parotid apparently inhibits PI resynthesis. Further studies should elucidate the exact causes and nature of these effects. (M. D. Dibner is a visiting scientist from E. I. du Pont Glenolden Laboratory).

153.2 SMALL INTENSELY FLUORESCENT (SIF) CELLS IN THE STELLATE GANGLION OF THE RAT AND GUINEA-PIG. V. A. Woodmansee, L. J. Lehman*, and D. G. Emery. Dept. of Zoology, Iowa State University, Ames, IA 50011.

Characteristics of a small intensely fluorescent (SIF) cell population in the stellate ganglion were examined in rats and guinea-pigs using the formaldehyde-induced fluorescence technique for monoamines of Loren et al. (Histochem. 52:223, 1977). Species differences can be observed in characteristics of the SIF cell populations.

Rats contained a larger population of SIF cells per ganglion (267) than did guinea-pigs (118). A larger number of SIF cells per milligram tissue was observed for rats (267/mg) vs. guinea-pigs (32/mg). Little difference was observed between the SIF cell populations in left vs right ganglion for either species.

SIF cells were categorized as Type I or Type II using the morphological characteristics described by Chiba and Williams (Cell Tiss. Res. 162:331, 1975). Table 1 shows these results. In rats, Type I cells had processes, and were sometimes mixed in clusters with Type II cells. Type II cells had no processes and were found in clusters with other SIF cells. Guinea pig SIF cell types were also mixed within clusters which were smaller than those found in rats.

Rat SIF cells were of larger diameter larger (10.4 μm) than guinea-pig cells (8.8 μm). Type I and Type II SIF cells did not differ in diameter in either species.

The data presented above for rats corresponds well with similar data obtained by others for the SIF cell population in the superior cervical ganglion of rats. Guinea pig data shows some deviation to other studies, particularly in the percentages of SIF cell types seen.

Currently, immunohistochemical studies are being carried out to determine the putative neurotransmitters in each of these SIF cell populations:

Table 1. SIF CELL TYPES IN THE STELLATE GANGLION

	Number (%) Type I Cells		Number (%) Type II Cells	
Ganglion	Rat	Guinea Pig	Rat	Guinea Pig
1L	21(10%)	44(37%)	188(90%)	75(63%)
2R	57(16%)	19(16%)	304(84%)	99(84%)
3L	16(5%)		295(95%)	
4L	7(7%)		88(93%)	
5R	21(12%)		156(88%)	
6L	66(13%)		429(87%)	
7R	28(13%)		194(87%)	
X	(11%)	(26%)	(89%)	(74%)

Supported by a grant from the American Heart Association.

153.3 CATEGORIES OF AXONS IN THE FELINE ANSAE SUBCALVIAE. D.G. Emery. Dept. of Zoology, Iowa State University, Ames, Iowa 50011.

In most cats three major sympathetic nerves arise from the stellate ganglion. The anterior and posterior ansae subclaviae pass around the subclavian artery and join near the middle cervical ganglion, and one or more nerves arise from the caudal margin of the stellate ganglion and innervate the heart and other thoracic viscera. Previous studies have shown that the major of these nerves, the inferior cardiac nerve, contains mostly unmyelinated postganglionic and myelinated sensory axons. One objective of this study was to determine if either of the ansae subclaviae contain populations of unmyelinated sensory axons, which are known to exist and to function as pain pathways to the heart.

Sections of the ansae were examined by electron microscopy to determine the number of axons. Dorsal root ganglionectomies and ventral rhizotomies caused selected populations of axons to degenerate. By counting the number of surviving axons the composition of the functional categories of axons was deduced.

The results (Table I) show that the posterior ansa is very similar to the inferior cardiac nerve, consisting of 80% unmyelinated, mostly postganglionic axons and myelinated sensory and postganglionic axons. The anterior ansa also contains many unmyelinated axons (60%), the greatest part of which (60%) are postganglionic. The remainder includes many unmyelinated sensory axons (26%). The myelinated fibers are roughly evenly divided amongst preganglionic, postganglionic and sensory categories. It was also shown that most of the largest myelinated axons in both ansae degenerate after dorsal root ganglionectomy and are thus sensory. Autoradiography of sections of the anse and inferior cardiac nerve after injection of ^{3}H-leucine into dorsal root ganglia revealed labelled myelinated and a few labelled unmyelinated axons. Thus it seems that all the nerves from the stellate ganglion contain some unmyelinated sensory axons, but only the anterior ansa has a large number.

Table I. Average Numbers of Axons in the Ansae Subclaviae

	Anterior Ansa		Posterior Ansa	
Condition	#Myelinated	#Unmyelinated	#Myelinated	#Unmyelinated
1. Normal	3808	5505	2126	8258
2. Pre & post gangl.	2529	5241	858	11267
3. Postgangl.	1109(29%)	3285(60%)	808(38%)	6753(82%)
4. Sensory (#1-#2)	1279(34%)	1413(26%)	1268(60%)	0
5. Pregangl. #1-(3+4)	1420(37%)	807(15%)	50(2%)	1505(18%)

Supported by a grant from the American Heart Association

153.4 SEROTONERGIC NEURONS OF THE SMALL INTESTINE: DISTRIBUTION AND INTERACTIONS WITH OTHER NEURONS. S.M. Erde, M.D. Gershon, D.L. Sherman*. Dept. Anatomy and Cell Biol., Columbia Univ. P&S, New York, NY 10032.

Serotonergic neurons have recently been shown to be present in the enteric nervous system (ENS). We have studied the distribution of these neurons in the guinea pig small intestine. In addition, interactions between serotonergic, noradrenergic and other neurites in the myenteric plexus were analyzed. Serotonergic neurons and neurites were demonstrated by light and electron microscopic radioautography following incubation with ^{3}H-serotonin (^{3}H-5-HT; 0.5uM) in the presence of desmethylimipramine (10 nM) or 5-hydroxydopamine (1.0mM; 5-OHDA) to prevent uptake of ^{3}H-5-HT by noradrenergic axons. When $NaMnO_4$ (3%) was used for postfixation instead of OsO_4, simultaneous recognition of serotonergic (radioautographically labeled) and noradrenergic elements (50nm dense cored vesicles loaded with 5-OHDA) was possible. The density of innervation was estimated quantitatively by measuring uptake of ^{3}H-5-HT or ^{3}H-norepinephrine (^{3}H-NE) as a function of distance down the bowel. Serotonergic neurons were found to comprise about 3% of the neurons of the myenteric plexus. Essentially all serotonergic perikarya are situated at the periphery of a ganglion and expose a surface to the surrounding basal lamina. Bundles of serotonergic axons run in interganglionic connectives and ramify around neurons in innervated ganglia. Some ganglia receive a dense serotonergic innervation, some receive almost no serotonergic innervation, and some have densely innervated and non-innervated portions. There is thus a pronounced non-equivalence of ganglia. While the density of the serotonergic innervation seemed to vary widely between adjacent segments of small intestine, no change was detected in density as a function of distance from pyloric to ileocolic sphincters. In contrast, the noradrenergic innervation showed a proximo-distal decline in density. Axo-axonic interactions between serotonergic and other elements including noradrenergic and putative cholinergic axons occur and apparent noradrenergic synapses on serotonergic perikarya were also found. Serotonergic synapses are most frequently axo-somatic but axo-dendritic contacts occur as well. These observations indicate that the ENS is more complex than previously imagined. Individual myenteric ganglia differ from one another and enteric neurons interact with one another in a variety of ways. The significance of the innervation of serotonergic neurons by noradrenergic terminals remains to be established. Supported by NIH grant NS12969.

153.5 POTASSIUM DICHROMATE LOCALIZATION OF CHROMAFFIN ORGANS ASSOCIATED WITH THE INFERIOR MESENTERIC GANGLION (IMG). J.A. Mascorro* (SPON: F.E. Dudek). Department of Anatomy, Tulane University School of Medicine, New Orleans, LA 70112.

Paraganglia (PG) represent small groups of adrenal medullary-like cells that appose most sympathetic ganglia and which show a chromaffin reaction for catecholamines. They are regarded as secretory because of similarities with the endocrine adrenal medulla in terms of morphology and catecholamine release following stimuli. Similar chromaffin cells (SIF cells) also occur within sympathetic ganglia and reportedly can inhibit or excite neurons by releasing catecholamines onto the nerve cell. Thus, it seems necessary to reexamine the apposition of chromaffin organs with sympathetic ganglia, and to search for vascular connections that could carry paraganglion catecholamines into the ganglion. The present work addresses this issue by locating PG in association with the IMG as well as by studying their morphology and topographical relation with the IMG.

Six adult cats were anesthetized and perfused with glutaraldehyde in phosphate buffer. The retroperitoneal tissue blocks were then treated with a potassium dichromate/glutaraldehyde combination (Mascorro, J.A., Tissue and Cell, 9:447, 1977) which produced a gross chromaffin reaction in organs apposed to the IMG and presumed to be true PG. The position and number of these "paraganglia" was noted and photographed macroscopically. IMG showing dichromated organs were processed for electron microscopy for ultimate verification and detailed study.

The IMG was located around the base of the inferior mesenteric artery (IMA) where the artery originated from the abdominal aorta. The ganglion proper appeared flattened, irregularly shaped or in strips on either side of the IMA. All ganglia possessed at least one associated paraganglion, and usually more; one IMG displayed five discrete chromaffin bodies, the largest being 1 mm in length. Histological study showed a connective tissue plane between the chromaffin bodies and IMG and, furthermore, that chromaffin cell groups were prominent within the ganglion. The ultrastructural features of paraganglion and intraganglion chromaffin cells were identical and included many blood vessels and catecholamine granules. Vascular routes between the two organs were not noted but should not be excluded based upon present samples. It is of paramount importance now to pursue the hypothesis that chromaffin cells lying next to the IMG, or residing within the ganglion, can release catecholamines into the ganglion environment.
(Appreciation is extended to Dr. J.T. Weber and NIH Grant EY03731 for support.)

153.6 A COMPARATIVE STUDY OF INTRINSIC PROPERTIES OF CAT SOLAR PLEXUS NEURONS. D.L. Decktor, P.R. Rogenes and W.A. Weems, Dept. Physiol. & Cell Biol., Univ. Texas Med. Sch. at Houston, Houston, Tx. 77025

Neurons located within sympathetic ganglia of the solar plexus can be classified according to 1) location, 2) rhythmic firing behavior, and in many instances, 3) efferent fiber pathway. In previous studies, we have described certain intrinsic properties of neurons located within left celiac ganglia (LCG), left renal ganglia (LRG) and superior mesenteric ganglia (SMG). Within each of these ganglia efferent neurons having axons in specific nerve trunks were identified. Yet, no systematic comparison of intrinsic properties between solar plexus neurons classified as described above has been performed. The purpose of this study was to provide such a comparison to determine if these neurons are relatively homogeneous with respect to their intrinsic properties or whether the total cell population is comprised of a number of distinct subgroups whose intrinsic properties differ. Solar plexus ganglia (LCG, LRG and SMG) were studied in vitro. Stimulating electrodes were placed on nerves of the celiac and mesenteric plexus and the left renal nerves. Transmembrane potentials were monitored by intrasomatic electrodes. Input resistance and time constant were measured for each neuron. From these values total input capacitance was calculated. The amplitude and duration of the afterspike hyperpolarization (ASH) were measured and the rhythmic firing mode of the neuron assessed. Efferent neurons were identified by antidromic activation. Significant differences in intrinsic neuronal properties were observed between neurons of the LRG and SMG ganglia. LRG neurons exhibited higher input resistances (mean value, 27.9 MΩ) and lower input capacitances (mean value, 645 pF) than SMG neurons (18.3 MΩ and 1045 pF respectively). Mean values of input resistance and input capacitance for LCG neurons were 24 MΩ and 790 pF respectively. All neurons tested fired in a rhythmic firing mode. Seventy-one percent were phasic and 29% were tonic. Tonic neurons exhibited higher input resistances (mean value, 28 MΩ) and lower input capacitances (mean value, 631 pF) than phasic neurons. If it is assumed that specific membrane capacitance is constant, then differences in input capacitance between ganglia and between phasic and tonic neurons would reflect differences in neuronal geometry. The distribution of ASH durations obtained from SMG neurons was shifted to the right of distributions observed in the LCG or LRG. No significant differences were noted when intrinsic properties of subpopulations of efferent neurons within a particular ganglion were compared. It was concluded from these studies that neuronal geometry and the distribution of ASH durations vary significantly within and among ganglia of the solar plexus. Therefore solar plexus neurons cannot be represented as a a single population with similar intrinsic properties. (Supported by N.I.H. grant HL21351)

153.7 ENKEPHALINERGIC INHIBITION IN PARASYMPATHETIC GANGLIA OF THE CAT URINARY BLADDER. W.C. deGroat and M. Kawatani*(SPON: A.M. Booth). Dept. Pharmacology, Univ. Pittsburgh, Pittsburgh, PA 15261

Previous experiments have shown that dense networks of leucine enkephalin terminals surround the ganglion cells in the urinary bladder of the cat. These terminals which arise from preganglionic neurons in the sacral spinal cord are eliminated after transection of the sacral ventral roots or the pelvic nerve. The administration of exogenous leucine enkephalin inhibits transmission in bladder ganglia by a presynaptic mechanism which is in turn blocked by naloxone.

The present study was undertaken to determine whether endogenously released enkephalins might also modulate transmission in vesical ganglia. In previous experiments we have shown the repetitive stimulation (20-30 Hz,2-5 sec) of preganglionic axons in one branch of the pelvic nerve to the bladder produced a prolonged inhibition (20-40 sec) of the postganglionic discharge elicited by stimulation of preganglionic axons in another branch of the pelvic nerve. The possibility that this heterosynaptic inhibition was modulated by endogenous enkephalins was examined by determining its sensitivity to the opiate antagonist, naloxone.

Experiments were conducted in situ on cats anesthetized with chloralose. Heterosynaptic inhibition elicited by 2-5 sec trains of stimuli at 10-40 Hz ranged from 50-80% depression of the evoked response and persisted for 20-60 sec after the termination of the stimulus. Intravenous (20-50 µg/kg) or intraarterial (5-10 µg/kg) administration of naloxone reduced the duration and magnitude of the heterosynaptic inhibition in 8 of 9 experiments. On the average the duration of inhibition was decreased by 50% and the magnitude of inhibition by 30%. The effects of naloxone persisted for 1-2 hours. Naloxone did not alter the amplitude of the postganglionic potentials elicited by single shocks to a preganglionic nerve or the facilitation of transmission elicited by trains of stimuli at frequencies of 1-3 Hz. Naloxone did not reduce the homosynaptic depression of transmission following a single postganglionic discharge using the double shock technique. Heterosynaptic inhibition was not altered by other receptor blocking agents including adrenergic (dihydroergotamine), muscarinic (atropine), purinergic (theophylline) or GABAergic (picrotoxin).

In summary, the presence of L-Enk in preganglionic nerve terminals in bladder ganglia, coupled with the finding that naloxone, an opiate antagonist, reduces the ganglionic depressant actions of exogenous L-Enk as well as heterosynaptic inhibition suggests that L-Enk may be an inhibitory cotransmitter in the sacral parasympathetic outflow to the urinary bladder.

153.8 REFLEX ACTIVITIES IN PARASYMPATHETIC FIBERS INNERVATING THE PAROTID AND SUBMANDIBULAR GLANDS IN RABBITS. R. Matsuo* (SPON: K. Kusano). Dept. of Oral Physiology, Dental School, Osaka Univ., Osaka JAPAN

In order to evaluate the neural control mechanisms subserving reflex salivary secretion, the volume of secretion from parotid and submandibular glands was measured, and associated electrical activity in parasympathetic fibers serving these glands was recorded, in adult rabbits.

Electrical stimulations were applied to lingual and/or glossopharyngeal nerves in sympathetically decentralized animals. A more copious submandibular salivary secretion was induced by lingual nerve stimulation at 10 to 20 Hz than in the case of glossopharyngeal nerve stimulation. On the contrary, parotid secretion was strongly induced by glossopharyngeal nerve stimulation at 10 to 20 Hz.

Through functional single fiber analysis, about 50 % of the parasympathetic preganglionic fibers innervating the submandibular galnd responded to lingual nerve stimulation; about 40 % and 10 % of fibers responded to electrical stimulation of the infraorbital nerve and the glossopharyngeal nerve, respectively. As a result of repetitive electrical stimulation applied to the respective sensory nerve, parasympathetic submandibular fibers could be classified into three types according to the mode of reflex discharges. The fibers which increased their spontaneous discharges at 10 to 20 Hz stimulating rate were termed E-type fibers, while those which unchanged them were N-type fibers and those which decreased them were I-type fibers.

About 70 % of the postganglionic parasympathetic fibers innervating the parotid gland responded to stimulation of the glossopharyngeal nerve. The rest responded to stimulation of lingual and/or infraorbital nerves. Three modes of discharges (E-, N-, I-type) were also recorded in postganglionic fibers innervating the parotid gland. It was often observed that the effect of glossopharyngeal nerve stimulation was sustained for prolonged period after stimulation.

These results suggest that the afferent information via the lingual nerve plays an important role in reflex submandibular gland secretion, while information via the glossopharyngeal nerve plays an important part in reflex parotid gland secretion. The functional significance of three types of reflex discharges in parasympathetic fibers will be discussed.

153.9 IDENTIFICATION OF NEURONS IN THE SUPERIOR CERVICAL GANGLION INNERVATING THE CORNEA OF THE CAT. C. Frioni, C. Morgan, I. Nadelhaft, W.C. de Groat, P.J. Jannetta. Dept. Neur. Surg. & Pharm., Univ. Pitt. Sch. Med. & V.A. Hosp., Pittsburgh, PA

The cornea receives both sensory and autonomic innervation. In previous studies in the cat we have used HRP to trace sensory axons to the ophthalmic division of the trigeminal ganglion. The present experiments were designed to explore the autonomic innervation of the cornea. Catecholamines (CA) have been identified in the cornea of a variety of species. While the superior cervical ganglion (SCG) has been thought to be the primary source of this sympathetic input, direct evidence for this is lacking. We have therefore employed the following nerve tracing techniques to determine if neurons innervating the cornea were located in the SCG. In five cats HRP was injected into the substantia propria of the cornea. As a control for the possible spread of HRP and uptake by tissue surrounding the eye a similar amount of HRP was placed on the opposite, undamaged cornea. To identify possible neurotransmitters in SCG neurons in two cats, True Blue was injected into the substantia propria and sections of the SCG were processed for various peptides using FITC.

In the HRP experiments many cells were labeled on the experimental side (28-159; x = 64) and were located largely in the rostral half of the SCG. A few cells were lightly labeled on the control side, showing that some HRP can also be taken up either by the undamaged cornea or surrounding tissue. However, in view of the much larger number of cells on the experimental side it is reasonable to assume this HRP was taken up primarily by corneal axons. Cells were lightly labeled in the True Blue injected animals. Alternate sections of SCG were processed for leucine-enkephalin (LENK), methionine-enkephalin, substance P and vasoactive intestinal polypeptide (VIP). Only LENK and VIP were identified in these ganglia, but were not contained within True Blue labeled cells. LENK was seen in axon terminals presumably following dendrites and perikarya of ganglionic neurons. VIP was identified in cell bodies dispersed evenly over the ganglion.

We conclude the cornea in the cat does indeed receive an autonomic innervation from the SCG and that these axons are probably the source of CA terminals reported by others. Although the function of these axons in the cornea is unknown, it has been proposed that in other sensory systems, the sympathetic system modulates the sensitivity of receptors. A similar function may apply here. Since peptides were not observed in True Blue labelled neurons one might presume they are not transmitters here. However, caution must be used here as it is possible our methods were not sensitive to low levels of peptides. VIP has not been identified before in the SCG. While its function is unknown, it is possible that VIP is a cotransmitter in cholinergic neurons in the SCG.

153.10 SYMPATHETIC OUTFLOW IN THE PELVIC AND PUDENDAL NERVES. R.P.Kuo*, D.C.Kuo, W.C.deGroat, T.Hisamitsu*, K.B.Thor, and M.G.Backes*, (Spon: J.W.Yip)., Dept. of Pharmacol., Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

According to the traditional view, the sympathetic outflow to the pelvic viscera is contained in the hypogastric and lumbar colonic nerves, whereas the parasympathetic outflow is present in the pelvic nerve (PELN). However, in the course of a horseradish peroxidase (HRP) tracing study of the efferent component of PELN, we discovered that this nerve contained a large population of sympathetic postganglionic axons from the sympathetic chain ganglia. This report describes the distribution of sympathetic chain ganglion cells projecting to PELN and contrasts this population of cells with those which send axons into the pudendal nerve (PUDN), a nerve which also serves an important role in the regulation of pelvic visceral function.

In these studies neurons in the sympathetic chain ganglia of cats were labeled either with fluorescent dye (true blue) or with HRP applied to the central cut ends of PELN or PUDN. After appropriate time for transport the animals were perfused with fixative and the chain ganglia were removed and processed for tracer. A considerable variation was noted between animals in the anatomy of the chain ganglia at the sacral and caudal lumbar levels. Although it is generally accepted that in the cat there are seven lumbar and three sacral ganglia on either side we observed several instances where ganglia were either absent or adjacent ganglia were fused. The anatomy was more variable at the sacral level where fusion of the left and right chain ganglia was occasionally seen. A total of 3485-3850 paravertebral neurons (uncorrected for double counting) in the ipsilateral and contralateral L6-S3 sympathetic chain ganglia projected to PELN. The majority (82%) of labeled cells were concentrated in the ipsilateral S1 and S2 ganglia. Paravertebral contribution to PUDN could be discerned in L5-S2 ganglia, with the highest concentrations in L7 and S1. Of the total 5086-5624 cells counted, 5018-5349 were located ipsilaterally.

In conclusion, the present results demonstrate that PELN, which is generally thought to carry primarily parasympathetic efferent axons, has a considerable population of sympathetic postganglionic fibers from the sacral chain ganglia. The number of sympathetic neurons projecting to PELN was about 60% of that to PUDN, a somatic nerve which would be expected to contain a large sympathetic constitutent. It remains to be determined whether the postganglionic sympathetic component in PELN has different functional properties compared to those in the hypogastric and lumbar colonic nerves. Supported by NSF Grant PCM F906093 and NIH Grant 1-F32-NS07006.

153.11 INTRACELLULAR RECORDINGS IN THE INFERIOR MESENTERIC GANGLION OF THE RAT. D.L. Kreulen. Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.

The inferior mesenteric ganglion, a prevertebral sympathetic ganglion, is located in the mesentery adjacent to the inferior mesenteric artery which supplies the colon. The ganglion consists of two lobes of unequal and variable size joined by commisural-like fibers. The ganglia are innervated by a preganglionic nerve trunk, the intermesenteric nerve (IMN) which is joined along its course by lumbar splanchnic nerves. Two groups of post-ganglionic fibers project from the ganglia, the right and left hypogastric nerves (HGN) and the lumbar colonic nerves (LCN). Ganglia, with surrounding mesentery and blood vessels were removed from rats and pinned in an organ bath which was constantly superfused at 37°C. Nerve trunks were stimulated with bipolar platinum electrodes (pulse duration: 0.2-0.5 msec). The mean resting membrane potential was -55 mV ± S.E. 2.1 mV (n=29) and the mean rheobasic current was 0.18 nA ± S.E. 0.02 nA (n=15). The mean input resistance was 65 MΩ ± S.E. 14 MΩ (n=11).

Ninety-nine per cent of neurons tested received synaptic input via all of the nerve fibers. This input consisted of excitatory post-synaptic potentials (e.p.s.p.) and action potentials. The synaptic responses were often dispersed over a time period of 70-100 msec and some cells fired more than one action potential in response to a single shock. The average conduction velocities of the fastest conducting fibers were: IMN 1.3 M/sec ± S.E. 0.2 M/sec (n=26); HGN: 0.4 M/sec ± S.E. 0.03 M/sec (n=39); LCN: 0.3 M/sec ± S.E. 0.03 (n=7). When the colon was attached to the ganglion, continuous synaptic input was recorded in some of the ganglion cells. If the colon was distended by injecting Krebs solution into the lumen, the frequency and amplitude of e.p.s.p.'s increased. Cutting the LCN eliminated this synaptic input from the colon. Support USPHS NIH NS 14304 and HL 27781.

153.12 THE EFFECT OF VARIOUS ANESTHETIC AGENTS ON THE HISTOCHEMISTRY OF AUTONOMIC NERVES IN THE URINARY BLADDER. J.A. McConnell and G.S. Benson*. Dept. of Neurobiology and Anatomy and Div. of Urology, Univ. of Texas Med. Sch., Houston, TX 77125.

Previous studies of biopsied human urogenital tissue have indicated that anesthetics or other agents used during surgery cause depletion of norepinephrine (NE) in peripheral nerves. This hypothesis was tested with further analysis of human tissue and by examining nerve fibers in bladders of dogs treated with various anesthetic and/or preanesthetic agents, and these data compared with that from control animals.

Experimental tissue included 1) surgical biosies from patients undergoing cystectomy or ureteral reimplantation and 2) bladders from adult mongrel dogs anesthetized with one or more agents used routinely during human surgery, specifically sodium pentothal, atropine sulphate, scopolamine, ketamine, fluothane and ethrane. Control tissue came from unanesthetized human organ donors and from dogs anesthetized with sodium pentobarbital. (In previous work on rats, this anesthetic had no apparent effect on peripheral nerve NE.) Tissue samples were taken from each of the three regions of the bladder. Part of each sample was frozen and processed for NE-containing fibers with a glyoxylic acid (GA) cryostat method and for acetylcholinesterase (AChE) with a direct-coloring method. The remainder was processed for electron microscopy (EM), both with routine fixation and with glutaraldehyde-dichromate for the specific localization of NE.

Bladder tissue from humans and dogs given anesthetic agents demonstrated time-dependent changes in both the number and brilliance of GA-fluorescent fibers but not in AChE-positive nerves. Human tissue from short-term surgery (ureteric reimplantation) and dog tissue from the later stages (2-4 hrs) of pentothal or ketamine anesthesia, or the early stages of fluothane or ethrane anesthesia (1-2 hrs) had only slight decreases in fluorescent fibers compared to controls. Short-term anesthesia (1-2 hrs) with pentothal or ketamine and long-term anesthesia with fluothane and ethrane (2-6 hrs) caused significant decreases in the number of visualizable adrenergic fibers and in the level of their fluorescence.

With the EM, few small dense core vesicles were seen in long-term inhalant-anesthetized humans and dogs, but many were found in the control dogs. Human tissue from organ donors or short-term surgery had intermediate numbers of these vesicles.

These results suggest that anesthetics deplete peripheral NE either on a short-term basis from which the nerves recover (pentothal and ketamine), or over a longer period, the end point of which is yet undetermined (fluothane and ethrane).

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153.13 EFFECTS OF SYMPATHETIC ACTIVATION PROCEDURES UPON VENOUS MEMBRANE POTENTIAL IN INTACT RATS. W.J. Willems* (SPON: Z.J. Bosnjak), VAMC Research and Dept. Neurology, Medical College Wisc., Milwaukee, WI 53226.

The precise role of sympathetic activity in the control of vascular electrical parameters "in vivo" is poorly understood. This study was designed to document some effects of sympathetic activation upon intracellular potential of venular smooth muscle in intact rats with normal circulatory control. Following IP sodium pentobarbital anesthesia, tracheostomy, and femoral artery cannulation, male Sprague-Dawley rats were subjected to midline abdominal laparotomy. Mesenteric loops, with circulation and innervation intact, were suffused with physiologic saline solution at 37°C equilibrated with 5% CO_2-95% air. Intracellular membrane parameters were monitored in small mesenteric veins (300-600 um) with flexibly mounted glass microelectrodes (40-80 Megohms), using standard electrophysiologic techniques. The proximal portion of some mesenteric arteries and veins were cradled upon hook electrodes to permit stimulation with 0.7 msec square waves. The amplitude was adjusted to give a supramaximal constriction at 10 Hz frequency. Diameter response was monitored with Vickers split-image optics. Cutaneous pinch stimuli, blood withdrawal, and direct perivascular electrical stimulation were used to activate sympathetic input during intracellular impalements. During light periods of anesthesia, these procedures produced both measurable changes in both blood pressure and excitatory phenomena in single cells of venular smooth muscle. Excitatory phenomena observed after reflex activation included the initiation of transient electrical depolarizations (1-2 mV amplitude) which resembled excitatory junction potentials that others have reported to be elicited by "in vitro" perivascular stimulation. Waveforms resembling excitatory junction potentials also were found after averaging electrical responses to repeated single supraxaximal perivascular stimuli. More often, however, reflex activation of sympathetic activity was associaued with graded electrical depolarization or, when anesthesia was relatively deep, no measurable electrical change. Although venous electrical responses to sympathetic activation varied somewhat, the direction of the response, when present, was invariably excitatory. These measurements may help to clarify the role of excitatory electrical changes in neural control of mesenteric venular smooth muscle "in vivo". (Supported by NHLBI and VA Research grants).

153.14 MEDULLARY SINGLE UNIT RESPONSES TO CHORDA TYMPANI STIMULATION. J.S.Eisenman. Dept. of Physiology, Mt. Sinai School of Medicine, New York, N.Y. 10029.

As part of a program to study physiological activation of salivation in rats,I have attempted to localize and characterize neurons in the superior salivatory nucleus by electrical stimulation of the chorda tympani (CT).The CT was exposed in urethane anesthetized rats by a ventro-lateral approach,cut distally and placed on Pt-Ir stimulating electrodes (interelectrode distance, 2 mm).Extracellular unit responses were recorded using glass micropipettes (5-12μ tip) filled with Niagra Sky Blue,in 0.5M Na acetate.After ablation of one cerebellar hemisphere,the micropipette was placed,using surface landmarks,in the region of the nucleus,at or just caudal to the root of the facial nerve.A lateral angle of 32° was used to align the penetration with the dorsoventral axis of the nucleus.Unit responses were studied for latencies to CT stimulation and frequency following at intensities of 2X threshold.Responses which had invariant latencies and could follow two stimuli at 100/sec or more,were provisionally classified as antidromic or presynaptic axonal.Dye spots were placed iontophoretically to mark track positions and recording sites were localized histologically.In all, 112 units were studied,having a mean latency of 15.4 msec (range:2.7-37.1),SD=7.9.

Seventy units were localized to the area of the superior salivatory nucleus.Of these,5 (7%) formed a distinct group with short response latencies of 5.1-8.3 msec;the remainder had latencies of 11.0-37.1 msec.High frequency-following,100-450/sec,was found in 52 units.Units localized outside of the nucleus had similar latency and frequency-following ranges.However,of 42 such units,significantly more (18,or 43%) had short latencies (2.7-8.3 msec); 24 units had latencies of 12.5-31.6 msec.Based on location,latency and frequency-following,36 units were classified as antidromically activated preganglionic parasympathetic neurons.These had a mean latency of 20.2 msec,SD=6.2,frequency-following of 100-450/sec.The comparable group of 24 cells outside of the nuclear area,had a mean latency of 19.9 msec,SD=6.6.Since the short-latency responses were much more prevalent outside of the nucleus, they were not classed as salivatory neurons.These may represent activity originating from afferent fibers.

Measurement of conduction distances in several specimens,gave values of 15-17 mm.Mean conduction velocity for presumed preganglionic cells is, thus,0.8 M/sec;short-latency responses would have velocities as high as 6.3 M/sec.

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153.15 **ALTERATIONS IN THE CERVICAL VAGUS NERVE IN STREPTOZOTOCIN DIABETES IN THE RAT: A COMPUTERIZED MORPHOMETRIC STUDY.** **S.Y. Felten*, J.K. Szynal*, and D.L. Felten** (SPON: W.J. Anderson). Departments of Pharmacology and Anatomy, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

Diabetes was induced in 10 two month old male Sprague-Dawley rats by tail vein injection of 65 mg/Kg streptozotocin. All animals were diabetic at 48 hours after injection (blood glucose levels over 300 mg %) and remained diabetic until they were sacrificed at 6 months of age. The rats were anesthetized with Nembutal and perfused through the left ventrical with a solution of 1.5% glutaraldehyde - 0.5% paraformaldehyde in 0.08 M Sorensen's phosphate buffer. Cervical vagus nerves were removed and processed for routine electron microscopy. 1 um sections were cut, stained with toluidine blue, and observed and photographed (final magnification of 925X). Photographic montages were placed on a Summagraphics ID series digitizing tablet and analyzed using the QUANTAGRAPH computerized morphometric analysis system. Measurements were made of 1.) blood vessel number, diameter, cross-sectional area, and percent of nerve occupied by blood vessels; 2.) myelinated axon number, density, diameter, area, and percent of nerve occutied by axons; 3.) myelin profile diameter, area, thickness, and myelin area/axon area ratios; and 4.) unmyelinated axon number and unmyelinated/myelinated axon ratios.

Significant differences between control and diabetic rat vagus nerves ($p<0.05$ using a two-tailed unpaired student's t-test) include decreases in the total number of myelinated axons, and in the average diameter of myelinated axons. Large decreases were observed in the number of unmyelinated axons ($p<0.001$) in diabetic rats. In addition, the overall cross-sectional area of the cervical vagus nerve was significantly decreased.

153.16 CATECHOLAMINE SECRETION BY SAPONIN-SKINNED CULTURED ADRENAL MEDULLARY CHROMAFFIN CELLS. J.C. Brooks and S. Treml*. Marquette Univ. Sch. of Dent., Milwaukee, WI 53233

Isolated adrenal medullary chromaffin cells are receiving considerable attention in studies of the mechanism of catecholamine secretion. Secretion can be easily monitored for cells grown as monolayers in short-term culture in response to various secretagogues. However, the secretory apparatus itself is not accessible to direct chemical manipulation, exclusive of membrane effects, because of the semipermeable barrier function of the membrane. It is also difficult to distinguish between membrane effects, effects on the secretory apparatus itself, and interactions between the two. In an effort to gain direct access of drugs and proteins to the secretory apparatus we have used saponin to permeabilize the cells by the "skinning" process used with skeletal and smooth muscle cells.

In order to determine the minimum time required to skin the cells, monolayer cultures were exposed to 0.01% saponin for varying periods in the presence of the vital dye trypan blue. By direct visual observation, an optimal exposure time of 160 sec. was determined. The saponin treatment had no significant effect upon the total catecholamine content of the cells, nor did the cells appear different under phase contrast microscopy. Catecholamine and dopamine-beta-hydroxylase were released upon exposure of the treated cells to media with various calcium concentrations in the presence of exogenous Mg-ATP. Both Mg-ATP and calcium were essential for secretion. Secretion initiated by either of these agents was nearly complete after 10 min. of incubation. At this point calcium-induced catecholamine secretion was about 6-fold higher than that for cells incubated in calcium-free medium. Calcium-induced secretion was unaffected by mepacrine, an inhibitor of phospholipase A_2, or several drugs expected to influence secretion, including pargyline and N-ethylmaleimide. Reserpine caused a slight reduction of calcium-induced catecholamine secretion. Lactic dehydrogenase was released from cells by the saponin treatment and during the subsequent incubations, indicating that the treatment causes membrane permeability to molecules as large as protein.

The use of saponin to permeabilize chromaffin cells in culture appears to be a simple means for allowing the presentation of exogenous substances directly to the cell's secretory machinery. It should offer the opportunity to use chemical treatments and specific antibodies to cellular components to determine the role of these components in the secretory process. The techniques should also be applicable to a wide variety of cells known to secrete by an exocytotic mechanism.

153.17

WITHDRAWN

153.18 ADENOSINE MODULATION OF SYNAPTIC EFFICACY IN THE SUPERIOR CERVICAL GANGLION OF THE RAT. Barbara K. Henon and Donald A. McAfee. City of Hope Research Institute, Duarte, CA 91010.

Activation of adensine receptors in the CNS appears to cause inhibition of synaptic transmission by an unknown mechanism. Sympathetic ganglia also have adenosine receptors and we report here that the adenosine analog, 2-chloroadenosine, inhibits single evoked cholinergic EPSPs in the ganglion. EPSPs were recorded intracellularly from postganglionic neurons in the presence and absence of 2-chloroadenosine in the Locke solution. The average EPSP amplitude declined by 42 $\pm$ 9% of control in 10 μM (n=5) and 27% in 5 μM 2-chloroadenosine (n=2). Peak inhibition occurred in about 10 min and the effects were reversed in normal Locke solution within 30 min. There was no change in the input resistance of postganglionic neurons.

The same concentration of 2-chloroadenosine (10 μM) that inhibits EPSP amplitude was found to increase the number of synaptically evoked action potentials recorded intracellularly in response to a preganglionic stimulus train (20 Hz for .75 sec). The pulse train was delivered every 20 sec. Normally the response exhibits considerable synaptic depression with a few stimuli eliciting spikes and the remainder subthreshold EPSPs. In the presence of 2-chloroadenosine, more of the EPSPs exceeded threshold and the number of action potentials gradually increased to 181 $\pm$ 12% of control over a period of 10-12 minutes (8 trials on 4 cells). The facilitative effects of 2-chloroadenosine were reversed by washing in normal Locke solution for 20-30 min.

We have previously shown that adenosine inhibits Ca-dependent potentials by activation of adenosine receptors on the somata of postganglionic neurons (Henon and McAfee, 1979; Henon et al., 1980 Soc. Neurosci. Abst.). Our preliminary voltage-clamp studies have confirmed that adenosine inhibits the voltage sensitive Ca current. Inhibition of the single EPSPs by 2-chloroadenosine is consistent with this observation if it also inhibits Ca channels on presynaptic nerve terminals in the ganglion.

Increases in the number of intracellularly recorded spikes/train in the presence of 2-chloroadenosine is consistent with previous findings that adenosine reduces the amount of synaptic depression recorded extracellularly during repetitive preganglionic stimulation. Synaptic depression is also reduced by low Ca^{2+} in the medium and may result from the depletion of readily releasable ACh during the train. The mechanism by which 2-chloroadenosine inhibits single responses and yet facilitates repetitive activity in the ganglion is complex and is the object of future study. Understanding of this mechanism could lead to insights into the actions of adenosine in the CNS.

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153.19 ADRENALINE HYPERPOLARIZATION AND Na^+ PUMP IN SYMPATHETIC GANGLIA. P.E. Rafuse* & P.A. Smith. Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Ionic concentration gradients in neuronal tissue are maintained, at least in part, by an electrogenic, ouabain sensitive sodium pump. It has been suggested that the catecholamine induced hyperpolarization of both peripheral and central neurones may involve stimulation of the activity of this pump (Koketsu & Nakamura, Jap. J. Physiol., 26, 63, 1976; Kuba & Koketsu, Prog. Neurobiol., 11, 771, 1979; Phillis & Wu, Prog. Neurobiol., 17, 141, 1981; Segal, Brain Res., 206, 107, 1981). This hypothesis was examined using the sucrose gap technique to record responses from the IXth or Xth paravertebral sympathetic ganglia of the amphibians, *Rana catesbeiana* or *Rana pipiens*. Although adrenaline (Adr, 10μM-1mM) depolarized the membrane potential of 2 out of 10 preparations tested, the majority exhibited hyperpolarizing responses which were especially prone to desensitization. High concentrations of acetylcholine (10mM) produced a biphasic response, a depolarization followed by an afterhyperpolarization (ACh_{AH}) which has been shown to result from activation of the electrogenic Na pump (Smith & Weight, Nature, 267, 68, 1977). When the ACh_{AH} and hence the electrogenic Na^+ pump were inhibited by 10μM ouabain, application of Adr (20-200μM) still promoted membrane hyperpolarization. Under these conditions however, the rates of onset and offset of Adr hyperpolarization were somewhat slower than in the control situation. In another series of experiments, ganglia were stored overnight in K-free Ringer at 5°C. This treatment inhibits the Na^+ pump and causes accumulation of intracellular Na^+. When these preparations were examined in K-free Ringer, Adr (200μM) consistently produced a hyperpolarization of membrane potential. Re-administration of normal Ringer's containing 2mM K^+ for 1.5-2 min also promoted a transient hyperpolarization. This K^+-activated hyperpolarization (K_H) is due to the reactivation of the Na^+ pump which promotes hyperpolarization whilst accumulated Na^+ is disproportionately exchanged for K^+ (Akasu & Koketsu, Jap. J. Physiol., 26, 289, 1976). Following blockade of the K_H and hence the electrogenic pumping of Na^+ by 1μM ouabain, Adr still promoted a hyperpolarization of membrane potential. The mean amplitude of this response was 75.7 ± 17.5% of control (n=6). In two experiments the Adr hyperpolarization was actually enhanced in ouabain; in four others it was reduced. This reduction may have been attributable to desensitization. These results show that Adr can still promote hyperpolarizing responses when the Na^+ pump is apparently blocked. This observation questions, but may not necessarily invalidate, the hypothesis that Na^+ pump activation may be involved in the hyperpolarizing action of Adr.

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153.20 CATECHOLAMINES ENDOGENOUSLY MODULATE THE Ca^{++} DEPENDENT K^+ CONDUCTANCE WHICH UNDERLIES THE SLOW INHIBITORY POSTSYNAPTIC POTENTIAL (S-IPSP) IN MAMMALIAN PARASYMPATHETIC GANGLIA. P. Shinnick-Gallagher, M. Yoshimura*, and J.P. Gallagher, Dept. of Pharmacology, Univ. of Texas Med. Br., Galveston, TX 77550.

We have previously shown that the S-IPSP in cat vesical pelvic ganglia is due to direct muscarinic activation of a K conductance. We observed that superfusion with (1μM) phentolamine (15-20 min) enhances the amplitude of the S-IPSP in 75% of the cells. Superfusion of NE (10nM to 100μM) depressed the S-IPSP and the iontophoretically induced ACh hyperpolarization recorded in the same cell. These effects were usually accompanied by a membrane hyperpolarization (4-8mV) and conductance increase. When the membrane potential was voltage clamped NE still depressed the slow-inhibitory postsynaptic current (S-IPSC) and the ACh-induced outward current. NE did not affect the decay phase (τ) of the S-IPSC. At the 1μM concentration these effects occurred in every cell tested, even in cells where NE itself generated no outward current. NE shifted the concentration response curve for ACh in an uncompetitive manner. To test whether NE acted by blocking channels opened by ACh, we measured the voltage sensitivity of the NE effect on the ACh-induced outward current. The effect of NE did not appear to be voltage dependent. The reversal potential for the ACh-induced hyperpolarization was also not affected by NE. These data coupled with the lack of a NE effect on τ suggests that NE may not be exerting its primary effect by blocking channels directly. Since NE is known to inhibit Ca currents through activation of an alpha-adrenoceptor, the previous experiments implied that NE may be affecting a Ca mechanism and suggested that the S-IPSP may have a Ca component. The ACh hyperpolarization persists in a zero Ca/10mM Mg solution at a time when synaptic transmission is blocked. However, when the preparation is superfused with the zero Ca media for a longer time period, the ACh hyperpolarization is diminished in amplitude (20 min) and eventually blocked (40 min). Solutions containing more potent Ca antagonists, Cd (.3mM) or Mn (1mM), rapidly eliminated both the synaptic response and ACh hyperpolarization. Taken together (v.s.) these data suggest that the S-IPSP is due to a Ca dependent K conductance (g K_{Ca}) increase. The fact that the S-IPSP and ACh hyperpolarization can be observed under voltage clamp conditions that do not require depolarizing step suggests that the muscarinic hyperpolarization is due to an increased calcium conductance coupled to an ACh receptor, not to voltage dependent Ca entry, and that the resulting increase in intracellular Ca activates a potassium conductance. These data suggest that endogenous NE modulates the S-IPSP by inhibiting Ca entry through a postsynaptic alpha-adrenoceptor and probably not by blocking channels. (Supported by NS16228).

153.21 TOPOGRAPHY OF RAPHE-SPINAL PATHWAYS. J.B. Cabot and N. Bogan*. Department of Neurobiology and Behavior, S.U.N.Y. at Stony Brook, Stony Brook, New York 11794.

Vertebrate spinal cord contains major serotonergic terminations within dorsal horn(DH), ventral horn(VH) and the sympathetic preganglionic cell column(SPN). Neurons giving rise to these projections are located exclusively within the brainstem and include cells within the caudal pontine/medullary raphe complex(CPMR). The present series of anterograde ARG, retrograde HRP and immunohistochemical(IHC) studies, while focused primarily upon the identification of the specific CPMR subnucleus (i) projecting to the SPN, establish more generally the funicular trajectories and laminar termination patterns of avian CPMR-spinal pathways.

The pigeon (Columba livia) CPMR can be divided into at least 3 major subnuclei: obscurus(Rob), pallidus(Rpa) and magnus(Rmα,Rmβ). In normal and colchicine treated pigeons IHC staining with anti-5HT indicates that: (a) cells within each subnuclear region exhibit 5HT-like immunoreactivity; (b) among subnuclei the density of 5HT-like immunoreactive neurons is non-uniform, and none of the gigantocellular cells in Rmα stain positively; and (c)5HT neurons within CPMR give rise to axons localized to the dorsolateral(DL), ventrolateral(VL) and the superficial marginal layers of the ventral white matter (high cervical spinal cord).

Injections of HRP confined to the right hemicord in high cervical, brachial and thoracic segments result in labeling of neurons in all subnuclei (n=25). The laterality of labeling is complex: caudal Rpa, caudal Rmα, bilaterally; Rob, rostral Rpa, rostral Rmα, Rmβ, ipsilaterally. Combining thoracic HRP injections with bilateral dorsal quadrant lesions of high cervical cord clearly demonstrated that rostral Rpa, rostral Rmα and Rmβ give rise to spinally directed axons traveling principally in the DL and lateral white matter.

Injections of ^{3}H-amino acids throughout the rostrocaudal extent of CPMR confirm and extend the HRP data (n=10). The results suggest that neurons along the rostrocaudal axis of CPMR give rise to spinal laminar projections in the dorsoventral plane of the spinal grey. Specifically, injections: (a) confined to rostral Rpa, rostral Rmα and Rmβ result in terminal labeling primarily in the superficial layers of DH (I,II) and SPN;(b) centered in caudal Rmα and the rostral ½ of Rpa result in dense labeling of the intermediate spinal laminae, around the central canal, in SPN and VH; and(c) encompassing caudal Rpa reveal the presence of a distinctive "terminal ringing" of lamina IX motoneurons.

The combined HRP, ARG and IHC data strongly suggest that the funicular trajectories and laminar patterns of termination of CPMR neurons reflect, at least partially, the axonal projections and terminals of 5HT cells located in CPMR. (Supported by HL24103)

153.22 IS THE DIET-DEPENDENT EXCRETION OF 5-HYDROXYINDOLEACETIC ACID (5-HIAA) RELATED TO CHANGES IN THE INTESTINAL MUCOSA? J. L. Colmenares, V. Tortorici*, O. Restrepo* and A. G. Angulo-Colmenares. Servicios de Endocrinología y Anatomía Patológica. Hospital Militar "Dr. Carlos Arvelo", Caracas, Venezuela.

Previous work has shown that the synthesis and release of serotonin (5-HT) from the enterochromaffin (EC) cells is dependent on the amount of ingested protein and on the relation in the ingested protein between tryptophan (Tp) and the large neutral amino acids (Tp/LNAA; Colmenares, J. L. and R. J. Wurtman, Metabolism 28:820, 1979). Then a peripheral endocrine signal is generated by the EC cells in response to the diet. The question remained if the variations in 5-HT synthesis and release were due to changes in the number of intestinal mucosa cells which -contrary to serotoninergic neurons- vary with the diet.

Groups of Sprague-Dawley rats were fed diets I) 18% casein hydrolizate, Tp-free; II) 18% casein, 0.22% Tp; III) 8% casein, 0.10% Tp or IV) 18% casein supplemented with Tp up to 0.44% Tp; for 5 or 16 days. 24-hours prior to sacrifice urines were collected and 5-HIAA determined. Urinary 5-HIAA was related to Tp ingestion confirming previous reports. Plasma Tp, gut and blood 5-HT were measured. At 5 days they were decreased in group I; diet IV failed to produce increases even at 16 days. Diet III gave intermediate values. Diet II was used as a control.

The experiment was repeated for 5 days but prior to sacrifice rats were intubated with buffered formalin. Duodenal samples were embedded in paraffin and sections were stained with the diazo method for EC cells. The thickness of the mucosa, submucosa and muscular layers remained constant in all groups. A double-blind counting of the number of cells positive to the diazo staining showed a 60% decrease in group I, but there were no differences among groups II, III and IV.

In a third experiment rats were fed diets I or II for 5 days when they were intubated with 10 mg of a Tp solution or water. The following 3-hour excretion of 5-HIAA increased in the animals receiving Tp independently of the diet. Preliminary results indicate that only animals on diet I intubated with Tp showed an increase in diazo-positive cells.

These results suggest that the EC cells are saturated with 5-HT at high protein intake; that gut 5-HT decreases to mantain the circulating levels of 5-HT; and that the decreases in gut 5-HT during short term periods apparently are not due to decreases in mucosa thickness nor to the lack of the synthetic enzymes in the EC cells. An hypothetical model is presented to describe the EC cell system.

154.1 DOSE-DEPENDENT EFFECTS OF A γ-AMINOBUTYRIC ACID DERIVATIVE ON MEDULLARY AND PHRENIC RESPIRATORY NEURONS. P. M. Lalley, Dept. of Physiology, Univ. of Wisconsin, Med. Sci. Ctr.,Madison,WI 53706.

Baclofen administered intravenously can either stimulate or depress intercostal motoneurons, depending on the dose (Lalley, P.M., Br. Res. Bull. 5: 565, 1980). In this study, the effects of baclofen on medullary and phrenic neurons were investigated. Inspiratory activity recorded from the C_5 branch of the phrenic nerve increased following low doses of baclofen. Spike frequency within each inspiratory burst increased after lowest effective doses (0.5-1.0 mg/kg i.v.) without appreciable change in inspiratory duration (T_i) or expiratory silent period (T_e). After higher doses (1-4 mg/kg i.v.), T_i was either greatly prolonged, with little or no change in T_e, or the inspiratory discharge was continuous. Spike frequency at these higher doses was often reduced. Control phrenic nerve activity was largely reinstated by bicuculline (100-300 µg/kg i.v.). Extracellular and intracellular recording from phrenic motoneurons revealed increases in action potential frequency and T_i after 0.5-1 mg/kg, accompanied by depolarization. Higher doses (2-4 mg/kg i.v.) resulted in hyperpolarization, decreases in spike frequency and further prolongation of T_i. Medullary inspiratory neurons of the dorsal and ventral respiratory groups were stimulated by baclofen (0.5-2 mg/kg) and depressed by doses greater than 2 mg/kg. Expiratory neurons were depressed after doses of baclofen which increased phrenic nerve activity. When phrenic nerve activity was depressed by doses greater than 2 mg/kg, expiratory neurons sometimes discharged continuously. Microelectrophoretic application of baclofen (10 mM, pH 3) to medullary inspiratory neurons resulted in depression of firing at all effective current strengths (20-100 na). It appears that lower i.v. doses of baclofen increase inspiratory activity by suppressing an unidentified population of inspiratory-inhibiting neurons, whereas higher doses depress medullary inspiratory neurons and phrenic motoneurons.

154.2 RESPIRATORY DEPRESSION PRODUCED BY GLYCINE INJECTED INTO THE CISTERNA MAGNA OF THE CAT. R.A. Gillis*, A. Buller*, P. Hamosh*, A.M.T. Da Silva*, J.A. Quest* and J.R. Holtman, Jr. (SPON: W.E. Norman). Depts. of Pharmacology, Physiology and Pulmonary Medicine, Georgetown University Schools of Medicine and Dentistry, Washington, D.C. 20007.

The purpose of our study was to determine the effect of centrally administered glycine on pulmonary ventilation in the cat. Animals were anesthetized with - chloralose and trachael airflow was measured using a Fleisch no. 1 pneumotachograph. The flow signal was integrated to obtain tidal volume (V_t).

Administration of glycine (0.8-64.8 umol) into the cisterna magna of 7 animals resulted in a dose-related decrease in respiratory minute volume ($\dot{V}_e$). The highest dose tested (64.8 umol) resulted in a decrease in $\dot{V}_e$ from 454 ± 35 to 156 ± 44 ml/min ($p < 0.05$). This decrease was due primarily to a reduction in V_t which dropped from 31 ± 2 to 12 ± 2 ml ($p < 0.05$). The 2 largest doses tested, 21.6 and 64.8 umol, also produced a decrease in respiratory rate (f) from 14 ± 1 to 11 ± 1 breaths/min and 15 ± 1 to 12 ± 1 breaths/min ($p < 0.05$), respectively. Apnea occurred in 2 of 7 animals with the 64.8 umol dose. The respiratory depressant effects of glycine, over the dose range used in this study, appear to be localized to the CNS since intravenous glycine (64.8 umol) had no significant effect on respiratory activity.

In an attempt to determine the site of glycine to depress respiration when given by intracisternal injections, this substance was applied topically to the intermediate area of the ventral surface of the medulla using paired Perspex rings (W. Feldberg, Neuroscience 1: 427, 1976). Application of a dose of 13.3 umol of glycine produced apnea in 4 of 5 animals tested.

These results demonstrate that intracisternal administration of glycine causes pronounced respiratory depression. The CNS site of action for this effect may be the intermediate area of the ventral surface of the medulla. Supported by a grant from NHLBI (HL 29562).

154.3 β-ALANINE, TAURINE AND GLYCINE: EFFECTS ON CENTRAL RESPIRATORY REGULATION: P. Wessberg*, J. Hedner*, T. Hedner* and J. Jonason* (SPON: T. K. Harden). Dept of Pharmacology, Univ. of Göteborg, Göteborg, Sweden.

Several amino acids which normally occur in the CNS, like β-alanine, taurine and glycine, exert a general depressive influence on neuronal activity. In order to investigate the possible action of these amino acids on central respiratory regulation, we have studied the effects of intracerebroventricular (icv) injections on basal and CO_2 induced respiratory performance.

Halothane anesthetized rats with chronic icv catheters were studied in a whole body plethysmograph. The following respiratory parameters were registered: tidal volume (V_T), respiratory frequency (f), minute volume ($\dot{V}_E$), inspiratory time (T_I), expiratory time (T_E), respiratory time (T_{TOT}), "inspiratory drive" (V_T/T_I) and "respiratory timing" (T_I/T_{TOT}). Moreover, heart rate (HR) and mean arterial pressure (MAP) were recorded. The icv injections of β-alanine, taurine and glycine (0.01-1 mg) were given in the lateral brain ventricles.

All amino acids induced a decrease in f and V_T which was immediate and longlasting. In some animals periods of apnea were seen. Evaluation of the respiratory time intervals indicated that the effects on f were due primarily to a prolongation of T_E. Both V_T/T_I as well as T_I/T_{TOT} decreased after icv administration of the amino acids. Apart from the effects on basal ventilation the CO_2 induced respiratory response was blunted after β-alanine, taurine and glycine. A decrease in HR and MAP was seen after all agents.

In conclusion, this study shows that the putative amino acid neurotransmitters β-alanine, taurine and glycine have depressive effects on respiratory activity when administered by the central route. In spite of the high doses used it may be speculated that β-alanine, taurine and glycine may have a role in the central regulation of breathing during physiological or pathophysiological conditions.

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154.4 CARDIO-RESPIRATORY DEPRESSION PRODUCED BY CENTRALLY ADMINISTERED TAURINE IN THE CAT. J.R. Holtman, Jr., A. Buller*, A.M.T. Da Silva*, P. Hamosh* and R.A. Gillis*. Depts. of Pharmacology, Physiology and Pulmonary Medicine, Georgetown Univ. Schools of Med. and Dent., Washington, D.C. 20007

Studies in this laboratory have focused on the possible role of inhibitory amino acids in the central control of the respiratory and cardiovascular systems. In the course of these studies we have found that taurine(2-aminoethanesulfonic acid)depresses both respiratory and cardiovascular activity.

The effects of taurine(0.8-64.8 umol)were studied following intracerebroventricular (i.c.v.) and intracisternal (i.c.) injections in cats anesthetized with -chloralose. A femoral vein and artery were cannulated for systemic administration of drugs and measurement of blood pressure, respectively. A trachael cannula was inserted so that airflow could be determined by using using a Fleisch no. 1 pneumotachograph. The flow signal was then integrated to obtain tidal volume (V_t). Inspiratory and expiratory durations (T_i and T_e) were determined from rapid tracings of V_t.

After i.c.v. injectjon,taurine caused dose-related decreases in minute ventilation($\dot{V}_e$). With the largest dose tested (64.8 umol), $\dot{V}_e$ decreased from 492 ± 30 to 201 ± 43 ml/min ($p < 0.05$). The decrease in $\dot{V}_e$ was caused by a dose-dependent decrease in V_t which changed from 30 ± 2 to 12 ± 2 ml($p<0.05$). Apnea occurred in 2 of 7 animals given the 64.8 umol dose. Neither respiratory frequency (f) nor T_i and T_e durations were significantly changed. The cardio-depressant effect of taurine was evidenced by a dose-related decrease in mean blood pressure (MBP) with the highest dose producing a decrease from 112 ± 7 to 82 ± 12 mm Hg ($p < 0.05$).

Respiratory depression of a greater magnitude was seen when taurine was administered by i.c. injection. The maximal decrease in $\dot{V}_e$ was from 495 ± 59 to 64 ± 14 ml/min ($p < 0.05$), V_t from 27 ± 3 to 5 ± 1 ml ($p < 0.05$) and f from 18 ± 1 to 12 ± 2 breaths/min ($p<0.05$). Again T_i and T_e were not significantly changed. Apnea occurred in 5 of 6 cats receiving 64.8 umol taurine. In contrast to i.c.v. administration, taurine by i.c. administration did not cause hypotension. The cardio-depressant effects of taurine, over the dose range in this study, appear to be localized to the central nervous system since intravenous taurine (64.8 umol) had no significant effect on cardio-respiratory activity.

These data demonstrate that both i.c.v. and i.c. injections of taurine depress respiratory activity,while i.c.v. injections also lower MBP. From these findings, it appears that the site of action for taurine-induced hypotension differs from the site of action for taurine-induced respiratory depression.

154.5 PICROTOXIN PRODUCES CARDIO-RESPIRATORY STIMULATION BY BLOCKING THE VENTRAL MEDULLARY GABA SYSTEM. K. A. Yamada* and R. A. Gillis* (SPON: G. J. Blake). Dept. of Pharmacol., Georgetown Univ. Schs. of Med. and Dent., Washington, DC 20007.

We have previously reported that a tonically active GABAergic system influencing respiratory and cardiovascular function may exist at the intermediate area of the ventral surface of the medulla. Both GABA and muscimol were shown to act at this site to produce cardio-respiratory depression, while bicuculline was shown to stimulate ventilation and increase arterial pressure and heart rate. To test further for the existence of a GABAergic system at the ventral surface of the medulla, we evaluated the cardio-respiratory effects of another antagonist of GABA, namely picrotoxin. This agent was applied to the intermediate area of the ventral surface of the medulla (50-100 ug) of 5 chloralose-anesthetized cats while monitoring tidal volume (V_T), respiratory rate, blood pressure (BP), and heart rate (HR). These doses increased minute ventilation from 387 ± 30 to 476 ± 73 ml/min by increasing V_T from 26.7 ± 1.7 to 33.6 ± 2.2 ml ($p < 0.05$), as rate was unchanged. BP was increased from 161 ± 7 to 187 ± 10 mm Hg and HR from 224 ± 13 to 256 ± 14 beats/min ($p < 0.05$). Picrotoxin (50-150 ug) applied to the intermediate area of three chloralose-anesthetized cats which had become apneic after GABA administration re-established spontaneous breathing and reversed GABA-induced hypotension. These data are consistent with our hypothesis that GABA may be an important inhibitory neurotransmitter regulating pulmonary ventilation and cardiovascular activity from the intermediate area of the ventral surface of the medulla.

154.6 ACUTE SARIN TOXICITY: COMPARISON OF CENTRAL NERVOUS SYSTEM AND NEUROMUSCULAR EFFECTS. D.L. Rickett, N.L. Adams*, R.E. Foster, J.F. Glenn, W.T. Gregory*, T.C. Randolph*, and R.K. Traub*. Neurotoxicology and Experimental Therapeutics Branch, U.S. Army Medical Research Institute of Chemical Defense, APG, MD 21010.

Sarin (GB) is an organophosphorus anticholinesterase agent which is more susceptible to atropine and oxime therapeutic treatment than soman. This responsiveness to therapy is thought to be due to the relatively slow rate, compared to soman, at which GB-inhibited cholinesterase ages. Disagreement exists concerning both the sites and mechanisms of actions thought to be responsible for GB's toxicity. The present study was conducted to examine the acute toxicity of GB and to identify the relative significance of its actions on the central nervous system (CNS) and at the periphery. Using Dial-urethane anesthetized cats, recordings were made of medullary respiratory-related units, phrenic nerve discharge on the side contralateral to unit recording, electromyographic activity and contractions of the diaphragm leaflet innervated by the monitored phrenic nerve, airflow, blood pressure and electrocardiographic activity. Blood gases and expired CO_2 were also monitored. Sarin was administered via the femoral vein at a rate of one ml/min in a concentration of 3.0 µg/ml/kg. GB infusion was stopped when spontaneous respiration ceased, whereupon diaphragmatic neuromuscular blockade was tested by administrations of supramaximal stimulation of the phrenic nerve (10 & 100 Hz, 0.5 msec pulse, 0.5 sec duration) and artificial respiration was initiated. GB infusion was then continued at the previous rate but at three times the initial concentration and diaphragmatic responsiveness was tested at varying intervals. The results show that there was a loss of synchronous discharge of respiratory-related units. This disruption of normal neuronal activity preceeded GB-produced respiratory arrest and was maximally reflected as a loss of phrenic nerve discharge. At the time that spontaneous respiration ceased, tetanic contractions of the diaphragm could be elicited by phrenic nerve stimulation. It was not possible to establish the dose of GB required to block the diaphragm since in most cases, cardiac failure and loss of blood pressure precluded further infusion of GB. At the time of respiration arrest, $PaCO_2$ was within normal range while PaO_2 was depressed. The findings in this study strongly suggest that GB toxicity, like soman toxicity, is acutely mediated through a loss of central respiratory drive; this results from disruptions of normal patterned activity in respiratory-related unit discharge. GB also appeared to be more cardiotoxic than soman. Although the CNS actions of GB remain to be elucidated, our observations suggest that normal ionic conductances are altered.

154.7 TRH AND TRH ANALOGUES IN CENTRAL RESPIRATORY REGULATION. J. Hedner*, T. Hedner*, P. Wessberg*, D. Lundberg* and J. Jonason* (SPON: G. R. Breese). Dept of Pharmacology, Univ. of Göteborg, Göteborg, Sweden.

Recent immunohistochemical studies have shown that the tripeptide TRH has an extensive distribution in autonomic regulatory brainstem areas. As this area includes the structures involved in respiratory regulation, we have investigated the respiratory effects of TRH and TRH analogues administered centrally to anesthetized rats.

The rats were lightly anesthetized with halothane and studied in a whole body plethysmograph. The respiratory parameters: frequency (f), tidal volume (V_T), inspiratory time (T_I), expiratory time (T_E) and tidal respiratory time were studied. Moreover, minute ventilation ($\dot{V}_E$) and the quotients inspiratory drive (V_T/T_I) and respiratory timing (T_I/T_{TOT}) were calculated. Mean arterial blood pressure (MAP) and heart rate (HR) were continuously registered. Injections were given into the lateral brain ventricles or into the fourth ventricle according to a procedure earlier described (Hedner et al, 1981).

TRH resulted in a dose-dependent stimulation of f and $\dot{V}_E$ while V_T was slightly decreased after injection into the lateral as well as into the fourth ventricle. MAP and HR were not significantly changed in the dose range used (0.05-5 µg). All respiratory time intervals, T_I, T_E and T_{TOT}, were significantly shortened. Inspiratory drive increased while respiratory timing decreased. The response to a CO_2 challenge (5% or 10% CO_2 in O_2) was not changed compared to the control except for the f response which was lowered after increasing amounts of CO_2 in the inhalation gas mixture.

TRH in the deamidated form had no effect on the respiratory performance. DN 1417, a potent TRH analogue (Fukuda et al 1980) was found to be approximately ten times as potent as TRH as a respiratory stimulant even though the maximum f response did not reach the peak value seen after TRH.

The results indicate that TRH is a specific respiratory stimulant with an action within the CNS. Most probably the action is closely related to neurones involved in the basal regulation of breathing.

Fukuda et al. Chem. Pharm. Bull. 28, 1667, 1980.
Hedner et al. Naunyn-Schm. Arch. Pharmacol. 317, 315, 1981.

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154.8 MONOSYNAPTIC CONNECTIONS OF PULMONARY STRETCH RECEPTORS WITH P CELLS. D.B. Averill*, W.E. Cameron, and A.J. Berger. Department of Physiology and Biophysics, Univ. of Washington, Seattle, WA. 98195.

The nature of the synaptic connections between single slowly-adapting pulmonary stretch receptors (PSRs) and respiratory neurons in the tractus solitarius (TS) region have not been investigated. The present study examined these connections by utilizing the technique of cross-correlation analysis of the discharge intervals recorded for single PSRs and TS respiratory neurons. Data were obtained from 10 adult cats that were anesthetised, paralyzed, and artificially ventilated. The extracellular activity of single PSRs was recorded with a "floating" metal microelectrode placed in the nodose ganglion. The activity of single respiratory neurons was recorded with low impedance metal or glass microelectrodes placed in the medulla 0-2 mm rostral to the obex. The region of central termination for PSRs was identified by the occurrence of a terminal potential (tp) and a focal synaptic potential (fsp) in the spike-triggered average of extracellular field potentials. The only respiratory units studied were those within a limited distance (500 µm) from the observation of a tp and fsp. The discharge of the PSR and TS unit were recorded simultaneously on FM tape. Interspike intervals for both neuronal discharges were determined off-line; a minicomputer (LM^2) was used to construct cross-correlation histograms (CCH) for these simultaneously occurring interspike intervals. Respiratory units were classified as Iα, Iβ or P cells. In a few cases, tests were not performed to differentiate between Iα and Iβ cells; these units were classified as I_{nd}. Cross-correlation histograms were compiled for 14 PSR-TS unit pairs. The results of the crosscorrelation analyses are summarized below.

	CELL TYPE			
	P	Iα	Iβ	I_{nd}
Total	3	7	2	2
Monosynaptic Connections	3	0	0	0

The mean latency from PSR spike to the peak probability of the discharge for the P cells was 2.1 msec. This short latency along with the increased probability of discharge observed in the CCHs between PSRs and P cells indicate monosynaptic excitatory connections. The inability to demonstrate a change in probability of discharge for I cells may be explained by either 1) PSR afferents do not have synaptic connections with I cells or 2) cross-correlation analysis of neuronal events over repeated respiratory cycles (reference events>15K) may not be sufficiently sensitive to detect a weak synaptic connection between PSRs and I cells.

This work was supported by USPHS Grant 14857 and NSRA HL 06233.

154.9 HYPOXIA POTENTIATES REFLEX BRONCHOCONSTRICTION INDUCED BY LUNG DEFLATION IN DOGS. E.H. Vidruk* (SPON: Peter D. Spear). John Rankin Laboratory of Pulmonary Medicine, Department of Preventive Medicine, University of Wisconsin, Madison, WI, 53706.

Bronchomotor reflexes are known to play an essential role in controlling the caliber of both normal and diseased airways. How factors such as blood gas composition interact with these reflexes has not been explored. The purpose of this study was to determine whether alterations in arterial oxygen tension (PaO_2) affect the magnitude of reflex bronchoconstriction. Experiments were performed in anesthetized (i.v. chloralose, 100 mg/kg, and urethane, 500 mg/kg), paralyzed (i.v. Flaxedil, 2-4 mg/kg) and mechanically ventilated dogs. In each dog, the uppermost portion of the intact extrathoracic trachea was isolated in situ from the rest of the respiratory tract. Continuous monitoring of pressure within the isolated segment (P_S) provided an index of bronchomotor tone with an increasing P_S indicating bronchoconstriction and decreasing P_S, bronchodilation. Reflex bronchoconstriction was induced by stopping the ventilator during expiration (10-30 sec) and permitting the lungs to deflate spontaneously. This maneuver resulted in an increase in the P_S which was reversed upon reinstitution of ventilation and which returned to baseline within 30-60 sec. When this maneuver was employed during ventilation with normoxia (PaO_2, 72-90 mmHg), P_S increased by 1-23 cm H_2O (N=13). When repeated during ventilation with hypoxia (PaO_2, 29-49 mmHg), the increase in P_S ranged from 0.1-10 times greater than that with normoxia (N=13). By contrast, when done during ventilation with 100% O_2 (PaO_2> 200 mmHg) the increase in P_S was greatly attenuated (N=11; range 0-50% of normoxic results). Hypoxia and hyperoxia in most cases did not change baseline P_S from its normoxic value. Changes in oxygenation were accomplished by changing the concentration of O_2 in the inspired gas. Arterial CO_2 tension and pH remained virtually the same during hypoxia and hyperoxia as during normoxia. The reflex bronchoconstriction was essentially completely eliminated by bilateral cervical vagotomy (N=4). Bilateral neurotomies involving only the superior laryngeal nerves reduced the magnitude of the reflex by at least 50% (N=4). Bilateral neurotomies involving only the caudal pararecurrent nerves reduced the magnitude of the reflex by 50% in 3 dogs but was without effect in 1 dog.

The potentiating effect which hypoxia has upon bronchoconstrictor reflexes may be a mechanism underlying the responses of hyperreactive airways. These data also suggest that hyperoxia may be useful in preventing the full expression of reflex bronchoconstriction.

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154.10 SHORT TIME SCALE CORRELATIONS BETWEEN RESPIRATORY NEURONS OF THE CAT BRAINSTEM. L.S. Segers*, R. Shannon*, and B.G. Lindsey. (Spon: S. Saporta). Univ. So. Florida, Dept. Physiology, Tampa, FL 33612

Recent anatomical and electrophysiological studies have provided evidence for axonal projections between respiratory neurons of the medulla (nucleus ambiguus (NA), nucleus retroambigualis (NRA), area of the retrofacial nucleus (RFN)) and pons (nucleus parabrachialis medialis, Kolliker-Fuse nucleus). Preliminary studies of the temporal relationships of spike trains of simultaneously recorded pontine-medullary and pontine-pontine neuron pairs have been done. These studies, using cross-correlational methods, are designed to detect correlations indicative of synaptic interactions between neurons. Cats were decerebrated, vagotomized, paralyzed, and artificially ventilated. Neurons were recorded extracellularly using 2, 3, or 4 tungsten microelectrodes. Contralateral phrenic nerve activity was recorded. Eighty pairs of pontine-ventral respiratory group (NA, NRA) neurons have been studied. Short time scale correlations in this sample were rare: the cross-correlogram for one neuron pair had small peaks on both sides of the origin, similar to high-frequency oscillations observed by others. Twenty pairs of pontine-RFN neurons have been studied. Two of these pairs yielded evidence of synaptic interaction. A pair of expiratory (E) neurons contained a peak that straddled the zero lag, indicating the presence of a shared input to these two neurons. A pontine inspiratory (I) neuron and a RFN IE phase-spanning neuron exhibited a peak to one side of the zero lag, suggesting an excitatory projection from the IE to the I neuron. Eighteen pairs of pontine respiratory neurons have been analyzed. One of eight pairs of I neurons exhibited a peak that straddled the zero lag, indicating a shared input. One of eight pairs of pontine IE neurons also exhibited evidence of a shared input. These preliminary data 1) show that cross-correlational analysis is sufficiently sensitive to detect interactions between distant pontine and medullary neuron pairs and 2) suggest that interactions between medullary and pontine neurons are rare, "weak", or limited primarily to specific subgroups of respiratory neurons. These data are consistent with the antidromic studies of Bianchi and St. John.

154.11 TIMING OF THE RESPIRATORY CYCLE BY STIMULATION OF THE CENTRAL NUCLEUS OF THE AMYGDALA. R.M. Harper, R.B. Trelease* and R.C. Frysinger. Dept of Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024

Changes in respiratory pattern associated with different sleep-waking states or behavior reflect differences in the timing of transitions between inspiratory and expiratory phases of the respiratory cycle. The "pneumotaxic area" situated in the parabrachial pons is involved in the CNS control of respiratory phase switching, as indicated by both stimulation and lesion studies. The central nucleus of the amygdala (ACE) projects heavily to the parabrachial pons, as well as to medullary respiratory areas, and thus is a prime candidate for exerting forebrain control over respiratory cycle timing.

Anesthetized cats were prepared with bipolar stimulation electrodes in the ACE, together with EEG and EOG electrodes to assess state. Diaphragmatic electrodes were placed in the crura to record EKG and respiratory-related EMG. A pressure monitoring catheter was advanced into the aorta from the femoral artery. After 1 wk recovery, cardiorespiratory responses to both single pulse and short train electrical stimulation of the ACE were recorded in awake, undrugged, and unrestrained animals.

Short (300-500 msec) 90 Hz trains of 0.2 msec pulses produced rapid onset sustained inspiration and a rise in blood pressure. Single pulse stimulation during the expiratory phase resulted in an immediate switch to inspiration, and repetitive single pulses, close to the spontaneous respiratory rhythm, led to "paced" respiration (Fig 1); these responses occurred in the absence of a blood pressure rise. The effectiveness of a single pulse depended on its relationship to the respiratory cycle.

The ACE thus appears to be capable of altering the timing of inspiratory onset. This effect may be mediated through its anatomical relationship to the "pneumotaxic area" of the pons, or through its projections to the medulla. The widespread forebrain projections to the amygdala may thus provide considerable input from "higher" nervous structures to respiratory control systems.

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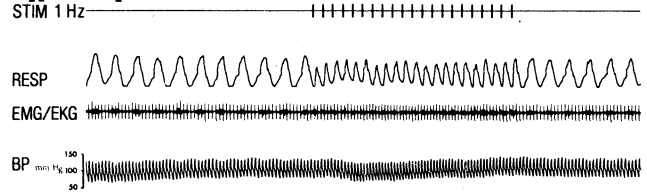

Figure 1. Repetitive single pulses of the ACE paces inspiration.

154.12 DISCHARGE OF DIAPHRAGM MOTOR UNITS DURING SLEEP. G.C. Sieck, R.B.Trelease, and R.M. Harper. Department of Anatomy and Brain Research Institute, UCLA, Los Angeles, Ca. 90024.

Diaphragm motor units are the final output of respiratory neural drive. Sleep states can markedly influence the activity of other respiratory muscles, but state influences on diaphragm motor control remains controversial. The present study examined the discharge of diaphragm motor units during different sleep-waking states in order to assess the changes in diaphragm neuromotor control. Adult cats were instrumented with electrodes for monitoring sleep-waking states (i.e., EEG, EOG, lateral geniculate nucleus activity and EKG). Pairs of insulated fine wires were inserted into costal and crural regions of the diaphragm. Recordings were obtained from unrestrained animals during quiet waking (AW), quiet sleep (QS) and rapid eye movement (REM) sleep. Motor unit signals were digitized (at 10kHz) and spike waveform and amplitude characteristics were examined. Autocorrelation histograms were calculated on discharge intervals of each motor unit. When the activity of two motor units was recorded simultaneously, cross correlation histograms were calculated to assess their discharge coupling. Repetitive patterns of motor unit discharge were observed in three general interval ranges: 1) 30-40 msec; 2) 60-70 msec; and 3) 90-110 msec. The slower periodicities (90-110 msec) were typical of crural motor units showing tonic discharge. The fastest periodicities (30-40 msec) were detected in motor units that were phasically, but inconsistently recruited with inspiration. Intermediate periodicities (60-70 msec) were detected in motor units that were phasically and consistently recruited with inspiration. During QS, periodicities were often attenuated and in some cases, the period duration slowed compared to AW and REM. Generally, recruitment was delayed during sleep states compared to AW. Most striking was the disappearance of activity of some motor units during REM sleep. During inspiration, the discharge rates of some motor units progressively increased (augmenting pattern). This augmenting pattern was slowed during QS compared to AW and REM. Cross correlations between motor unit discharges showed short-latency (<5msec) and longer latency periodic (25 to 70 msec) discharge coupling. Short-latency synchrony was not affected by state. Longer latency discharge coupling was usually attenuated during QS and the period duration was slowed compared to AW and REM. Cross-correlations further showed a constant order of recruitment of motor units during each state. These results demonstrate the pronounced influence of sleep-waking state on diaphragm motor unit recruitment and discharge.

Supported by HL 22418-04 and AHA 659 to GCS.

154.13 POSTURAL EFFECTS ON INTERCOSTAL MUSCLE ACTIVITY IN SLEEP. T. Dick, P. L. Parmeggiani* and J. Orem. Dept. of Physiology, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX 79430.

Intercostal muscle activity (IMA) has both respiratory and postural components. Some postural reflexes are suppressed in sleep. For example, righting reflexes are suppressed with the voluntary assumption of sleep postures, but we report here a dramatic, tonic, postural influence on the intercostal muscles in nonrapid eye movement (NREM) sleep.

IMA was studied in six freely moving cats. The animals had electrodes for recording the electroencephalogram, the electro-occulogram, and pontogeniculo-occipital waves as well as electromyograms from the neck and two intercostal muscles. During the 5- to 8-hr recording sessions, the animals were unrestrained within a shielded recording chamber. The data were recorded on a polygraph and an analog tape recorder. We noted the posture and movement on the polygraphic recording. We compared IMA recorded from the same muscle as the cat lay curled on opposite sides. In one position, the intercostal muscle was on the downward or convex side of the cat; in the other, the intercostal muscle was on the upward or concave side. Half-wave rectified and integrated IMA was summed over 9-s periods. A mean and standard deviation were calculated for 9-s periods while the cat lay in a given posture and stage of sleep. We tested differences between the means of IMA in the two positions using the t-test.

In NREM sleep, IMA was significantly greater in 87% (20 of 23) of the cases when the intercostal muscle was on the animal's concave rather than the convex side. This postural effect was strongest in intercostal muscles showing inspiratory activity (presumably the external intercostal muscles).

In REM sleep, the intercostal muscles, like many other muscles, are hypotonic or atonic, but some residual respiratory activity generally persists. In 69% of the cases, this residual respiratory activity was significantly greater when the muscle was on the concave side.

In conclusion, posture influences intercostal muscle activity in sleep. Cervico-labyrinthine reflexes may mediate the effects demonstrated here. Furthermore, slowly adapting, pressure receptors in the skin that inhibit ipsilateral but facilitate contralateral intercostal activity may be involved. The postural effect is often evident even in REM sleep.

154.14 PROLONGED ALTERATION OF RESPIRATORY CONTROL IN THE NEWBORN DOG AFTER CHRONIC MATERNAL HYPERCAPNIA. K.L. McGilliard*, S.E. Jones* and G.D. Olsen* (SPON: E.T. Iwamoto). Dept. of Pharmacology, Sch. of Medicine, Oregon Health Sci. Univ., Portland, OR 97201.

In previous studies, it was demonstrated that both infants and puppies chronically exposed to opiate analgesics *in utero* showed blunted ventilatory responses to CO_2 during the first few weeks of life (Olsen and Lees, J. Pediatr. 96:983, 1980; McGilliard *et al.*, Respir. Physiol. 47: in press). One possible explanation for altered respiratory control in opiate-dependent neonates is that the developing chemoreceptors were exposed to a high level of CO_2 due to the respiratory depressant action of opiates on the mother. Abnormal chemoreceptor development might then occur in a chronic hypercapnic environment. If so, exposure of the fetus to chronic hypercapnia in the absence of drugs should produce alterations in respiratory control similar to those which were opiate-induced. To test this hypothesis, a pregnant bitch was exposed to an environment of 5% CO_2, 21% O_2 from the 2nd week of gestation until parturition. Respiratory control was assessed in the offspring from birth until 6 weeks of age. In the first week of life, P_{ACO_2} and V_T of CO_2-exposed puppies were significantly lower and respiratory rate was significantly higher than control, while $\dot{V}_E$ was similar in the two groups. During the remaining 5 weeks of the studies, V_T, $\dot{V}_E$, $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ were lower than control. At the same time CO_2-response curves of CO_2-exposed puppies were significantly displaced to the right of control and slopes were decreased by at least 25%. Data obtained from 6-week-old puppies are shown below:

	Control	CO_2-exposed	
$\dot{V}_E$	420 ± 45 (SE)	340 ± 29	$ml \cdot min^{-1} \cdot kg^{-1}$
V_T	9.92 ± 0.39	7.47 ± 0.32	$ml \cdot kg^{-1}$
Rate	44 ± 5	46 ± 4	min^{-1}
P_{ACO_2}	38.2 ± 1.3	38.2 ± 1.0	mmHg
$\dot{V}_{CO_2}$	9.44 ± 0.62	7.70 ± 0.59	$ml \cdot min^{-1} \cdot kg^{-1}$
CO_2-response:			
Slope	42.4	26.3	$ml \cdot min^{-1} \cdot kg^{-1} \cdot mmHg^{-1}$
Position[1]	37.8	43.4	mmHg

[1] P_{ACO_2} when $\dot{V}_E$ = 400 $ml \cdot min^{-1} \cdot kg^{-1}$

The results suggest that *in utero* exposure to a hypercapnic environment produces significant alterations in ventilatory control in the newborn puppy. Since differences remained at the conclusion of the study, the possibility exists that prolonged or permanent disruption of normal chemoreceptor development has occurred. (Supported by USPHS, NIH Grants HD-10034, HD-07084 and HL-05918).

155.1 INFLUENCE OF FREEZE/THAW AND ACID DEPROTEINIZATION ON MEASUREMENT OF GABA IN CSF. T.N. Ferraro* and T.A. Hare, Thomas Jefferson Univ., Phila., PA 19107

CSF-neurotransmitter studies are becoming a popular method of characterizing certain neuropsychiatric disorders. Investigations of GABA levels in CSF have served as a prototype documenting the utility of this approach only after careful consideration has been given to a number of in vivo and in vitro factors which influence such determinations. Recently, two additional studies of in vitro variations influencing CSF GABA levels have been reported. Abbott et al. (J. Neurochem. 37: 1042, 1981), utilizing a radioreceptor assay, reported increases of CSF GABA levels following multiple freeze/thaw cycles. Using the confirmed ion-exchange fluorometric (I-E/F) procedure we examined the effect of multiple freeze/thaw cycles on CSF GABA levels (N=12). Results demonstrate that levels are not increased by freeze/thaw. Reported differences may be related to the release of substances interfering with the ^{3}H-muscimol binding in their assay.

Perry et al. (J. Neurochem. 38: 766, 1982), utilizing an I-E/F procedure, found that deproteinization with 0.5% sulfosalicylic acid (SSA) results in lower values for CSF GABA than those seen previously using the I-E/F procedure but following deproteinization with 5.0% SSA. They concluded that 5.0% SSA had produced elevated values via on-column hydrolysis of conjugated forms of GABA known to be present in CSF. We have systematically evaluated the effects of final SSA concentrations ranging from 0 to 10% in CSF. Prior to treatment, CSF was placed in a boiling H_2O bath for 2 min. to avoid enzyme mediated increases of GABA (Arch. Neurol. 38: 491, 1981). SSA concentrations of 0, 0.05, 0.1, 0.5 and 1.0% correlated linearly with CSF GABA values ($r = 0.99$, $p < .001$). However, concentrations greater than 1.0% produced a plateau revealing GABA values to be 1.6-fold higher than those of acid-free aliquots. Comparison of acid-free and plateau values by linear regression revealed a highly positive correlation ($r = 0.99$, $p < .001$ N=8). Thus, GABA in CSF evidently exists as free and loosely bound pools. Assuming an equilibrium between these pools, measurement of strictly free GABA would appear less likely to reflect alterations of CNS function. Therefore, it seems preferable to deproteinize CSF with final SSA concentrations greater than 1.0%.

155.2 A CENTRAL CHOLINERGIC DEFICIT IN DIETARY THIAMIN DEFICIENCY Gibson, G.E., Pelmas, C.J.* and Blass, J.P., (SPON: F. Plum) Cornell Medical College, Burke Rehabilitation Center, White Plains, New York 10605.

Thiamin deficiency impairs short-term memory in man and thus may be a useful model of the metabolic encephalopathies that lead to dementia. Previous studies demonstrated that the combination of pyrithiamin poisoning and dietary thiamin deprivation decrease the performance of rats on an elevated taut string (i.e. the tight rope test). About 40% of the treated animals had reduced scores within 2 days and severe neurological deficits by day 12 (e.g. ataxia, loss of righting reflex, death). Pharmacological manipulations of this impaired behavior suggested a central cholinergic muscarinic deficit in the early stages of thiamin deficiency. To determine whether these changes were due to an action of pyrithiamin that was unrelated to thiamin metabolism similar studies have now been done on animals with dietary thiamin deprivation.

Male Wistar rats (55-65 g) received either a thiamin deficient diet ad lib. or a complete diet (ad lib. and pair fed controls). Animals in the two control groups maintained tight rope test scores of 11 for 25 days. The thiamin deficient animals divided into a responding (i.e. scores decreased more than 4 points for 2 or more consecutive days) and a non-responding group. In 5 experiments 33% of the thiamin deficient animals ($n = 75$) were responders whose performance was significantly ($P<0.05$) lower than nonresponders after 6 days of treatment. Other neurological signs such as ataxia or loss of the Wooley-White reflex were not apparent until day 21.

Injections of thiamin or cholinergic agonists partially ameliorated these deficits. Thiamin increased scores by 2.1 ± 0.2 ($n = 10$). The cholinesterase inhibitor physostigmine (2.4 ± 0.2; $n = 36$) and nicotine (2.3 ± 0.6; $n = 12$) partially ameliorated the deficit due to thiamin deficiency. The beneficial effect of physostigmine was blocked by the central nicotinic blocker mecamylamine (0 ± 0.13; $n = 15$) and the muscarinic blocker atropine (0.1 ± 0.2; $n = 19$), but not by the peripheral muscarinic blocker methatropine (2.1 ± 0.4; $n = 15$).

Thus, dietary thiamin deficiency leads to similar behavioral changes as pyrithiamin poisoning although neurological symptoms occur only after longer treatment periods in the dietary model. Furthermore, pyrithiamin caused a central muscarinic lesion, but dietary thiamin deficiency lead to a central, cholinergic muscarinic and nicotinic deficit that appears behaviorally important. Supported in part by grants NS 15125, NS 16997, the Burke Foundation, The Brown & Williamson Tobacco Company and the Will Rogers Institute.

155.3 THE BINDING OF ^{3}H-GABA TO SYNAPTIC MEMBRANES OF SPONTANEOUSLY HYPERTENSIVE RATS. Godfrey Tunnicliff* (SPON: G.K. Matheson). Laboratory of Neurochemistry, Indiana University School of Medicine, Evansville, IN 47712.

Evidence that central nervous system GABA mechanisms are involved in the regulation of blood pressure comes from various sources. For instance, the administration of GABA or certain of its agonists either directly or intracerebrally produces decreases in arterial pressure. Bicuculline can reverse this effect. On the other hand, sub-convulsive doses of antagonists of GABA tend to cause an elevation of blood pressure. In light of these observations both sodium-independent and sodium-dependent binding of ^{3}H-GABA to synaptic membranes prepared from the brain of spontaneously hypertensive rats (SHR) were measured and the results compared to those obtained from Wistar-Kyoto (WKY) controls.

The greatest effects were seen in the cerebral cortex and the data are shown in Table 1:

Table 1. ^{3}H-GABA Binding to Cortical Synaptic Membranes.

Strain	n	Na^+-independent	Na^+-dependent
WKY	5	2.5 ± 0.2	81 ± 9.5
SHR	5	1.8 ± 0.2	62 ± 7.0

Binding units are pmol/mg protein.

A 28% decrease in sodium-independent (receptor) binding and a 23.5% decrease in sodium-dependent binding was obtained in the hypertensive animals. A Scatchard analysis of the binding data revealed that in both cases the reduction in binding was the result of fewer binding sites rather than to differences in affinity constants.

These results are consistent with the evidence that a reduced GABA neuronal activity is related to increases in blood pressure and possibly to the development of hypertension.

155.4 β-ADRENERGIC RECEPTOR COUPLED ADENYLATE CYCLASE IN NORMAL AND DYSTROPHIC CHICK PECTORALIS. J. E. Steiss, B. L. Raney*, R. Dulos* and P. V. Sulakhe. Dept. of Physiol., Col. of Med., Univ. of Sask., Saskatoon, Canada, S7N 0W0.

This study was undertaken to determine whether muscle sarcolemmal bound, β-adrenergic receptor-linked adenylate cyclase system is affected in the very early stages of muscular dystrophy. We chose the genetically dystrophic chick as a model in which the superficial pectoral muscle becomes dystrophic around the post-hatch age of 4 weeks. We investigated the activities of adenylate cyclase (AC) in the absence and presence of AC activators (NaF, Gpp(NH)p and isoproterenol), the number of receptors (β-AR) by using [^{3}H]-dihydroalprenolol (DHA) binding assay and cholera toxin dependent ribosylation of G/F proteins that mediate the coupling between β-AR and AC. Pectoralis (PT) was obtained from embryos (normal (N) and dystrophic (D)) incubated for varying periods (11 d to 19 d) and from hatched chicks (1 d to 14 d). The following major observations were made: Basal AC of N-PT, prior to hatching, was greater (2- to 3-fold) than of D-PT; post-hatch basal AC activities of N- and D-PT were similar. On the other hand, fold-stimulations by NaF, Gpp(NH)p and isoproterenol + Gpp(NH)p of the basal AC were greater for D-PT compared to N-PT at various pre- and post-hatch periods examined. The total number of β-AR in N- and D-PT steadily increased, reaching a plateau around 6 d post-hatch. There was no significant difference in the receptor number (expressed as fmol/mg protein) between N- and D-PT except at 14 d post-hatch, in which case D-PT had a greater number of β-AR. Two polypeptide regions corresponding to 55K and 50K dalton were labelled following incubation of muscle homogenate with ^{32}P-NAD and cholera toxin. This was examined in 19 d embryos and 14 d hatched chicks. ADP-ribosylation of both polypeptide regions was higher (2- to 3-fold) in D-PT relative to N-PT. We concluded that the observed greater stimulation of AC in D-PT by NaF, Gpp(NH)p and isoproterenol results from either an increased amount of G/F coupling proteins or greater efficacy of coupling between the β-AR and AC. Our results also show that the G/F protein-requiring coupling process is affected at early stages of muscle development in the genetically dystrophic chick.

JES is a Post-Doctoral Fellow of MRCC; this work is supported by a grant from MDAC to PVS.

155.5 ADRENERGIC, OPIATE AND BENZODIAZEPINE RECEPTOR BINDING STUDIES IN DYSTROPHIC MOUSE BRAIN. M. Wilkinson, Diane Wilkinson* and R. Bhanot. Depts. Physiol. Biophys. & Obst. Gynaecol., Dalhousie University, Halifax, Nova Scotia, Canada.

In addition to muscular symptoms, myotonic dystrophy is characterised by CNS abnormalities such as gross structural changes, disturbances in sleep-related growth hormone secretion, and depressive illness. In dystrophic (dy) mice, spontaneous seizures and unusual calcium ion accumulation by brain mitochondria have been described. We have now investigated whether the CNS of dy mice demonstrates changes in neurotransmitter receptor binding when compared with the brains of control mice. We examined brain tissue from male 129/ReJ-dy (5-7 weeks old) and female 129 $B6F_1$/J-dy (6 months old) mice, together with their respective controls. Crude P_2 membranes were prepared by standard methods. Receptor binding assays were performed as reported (Wilkinson & Grovestine, Can. J. Physiol. Pharmacol. 59 504, 1981). β-adrenergic ([^{3}H]-dihydroalprenolol) binding was significantly reduced (Scatchard analysis) in whole brain (less cerebellum; -37%), cerebral cortex (-18%) and cerebellum (-35%) of male dy mice. Whole brain binding in female dy mice was reduced by 24%. There was no change in binding affinity (K_D) in any brain area. Benzodiazepine ([^{3}H]-flunitrazepam) binding sites in whole brain and cerebellum of male and female dy mice appeared normal. In addition, the stimulatory effects of GABA (10^{-4}M) on benzodiazaepine binding was identical in dy and control membranes.

Opiate ([^{3}H]-naloxone) binding was also unaffected in whole brain of male 129/ReJ-dy mice (5 weeks old) and in forebrain (less cerebellar and cerebral cortex) of female 129 $B6F_1$/J-dy mice (3 months old). In view of the changes observed in β-adrenergic binding sites we have also examined the binding of an α-adrenergic ligand ([^{3}H]-dihydroergocryptine). Single point assays (1.5nM) revealed that in forebrain of 129 $B6F_1$/J-dy females the number of α-adrenergic sites was reduced by 30% whereas in cerebral cortex the number was unchanged.

In conclusion, there appears to be a specific reduction in the number of adrenergic binding sites in dystrophic mouse brain. This alteration may be related to the known abnormally high excretion of catecholamines. Our results reemphasise the need to study extra-muscular membrane sites in this disease.

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155.6 A SPONTANEOUS AND IRREVERSIBLE ANIMAL MODEL RESEMBLING HUNTINGTONS DISEASE (HD): BEHAVIORAL AND BIOCHEMICAL CORRELATES. A. Hitri*, R.L. Borison and B.I. Diamond, Psychiatry Department, Medical College of Georgia and Downtown V.A., Augusta, GA, 30912.

We and others have previously shown that the administration of β,β-iminodipropionitrile (IDPN) to various animal species for seven days produces head and neck choreatic movements. These movements resemble those observed in hyperkinetic movement disorders and are preferentially antagonized and exacerbated by dopamine antagonists and agonists respectively. In the present studies we examined the underlying biochemical correlates of this behavior. Male Sprague-Dawley rats (200-250 g) were administered IDPN (75 mg/kg, i.p.) daily for seven days and behavior quantitated over the ensuing month. Rats were sacrificed, brains removed, and the caudate nucleus dissected out. Striatal tissue was assayed for dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3MT) and homovanillic acid (HVA) levels spectrofluorometrically, using the technique of Westerink and Korf (1975). DA receptor binding studies (using tritiated spiroperidol as the ligand) were also measured in striatal tissue by the modified method of Fields et al. (1977). Seven day IDPN administration to rats produced head and neck choreatic movements that lasted until sacrifice (one month), and in other animals at least for three months. These movements were persistent and spontaneous during this period and disappeared during sleep. Striatal DA, DOPAC, 3MT and HVA levels were significantly decreased from saline control animals. However, DA release, as measured by the 3MT/DA ratio, was increased over control values. The spiroperidol binding to striatal tissue in control animals exhibited two binding sites, one with a K_d of 103 pM and the other with a K_d of 1.62nM. The number of receptors associated with the high affinity site was 14.9 fmoles/mg tissue, and the low affinity site contained 67.5 fmole/mg tissue. IDPN treatment eliminated the specific binding for the low affinity site and significantly reduced the number of receptors at the high affinity site (1.08 fmole/mg tissue). The loss and reduction of DA receptors, together with the increased release of DA (although levels are falling), resemble the biochemistry of the DA system in HD, as well as much of that observed in the kainic acid model of HD. However, the kainic acid model fails to pharmacologically mimic the behavior of HD due to the necessity of using a DA agonist, and therefore appropriate pharmacologic testing may be misleading; this problem appears to be avoided in the IDPN model, suggesting its greater suitability as a pharmacologic model.

155.7 DOPAMINE RECEPTOR BLOCKADE OF AMOXAPINE: COMPARISON OF GC 8-OH AMOXAPINE PLASMA LEVELS AND DOPAMINE RADIORECEPTOR ACTIVITY. A.L.C.Pottash[1], T.E.Barker*[2], F. Goggans*[2], D.M.Martin*[1], A.W.Caswell*[1], M.S.Gold[1]. Psychiatric Diagnostic Laboratories of America and Research Facilities, Fair Oaks Hospital, Summit, NJ;[1] and Psychiatric Institute of Fort Worth, Fort Worth, Texas.[2]

Amoxapine is a new subclass of tricyclic antidepressant designated chemically as 2-chloro-11-(1-piperazinyl) dibenz (b,f)(1,4) oxazepine. Antidepressant activity of amoxapine is related to the blockade of Norepinephrine and Serotonin. There are two major metabolites, 7-OH-Amoxapine and 8-OH-Amoxapine and it appears that these metabolites are responsible, more so than the parent compound, for pharmacological effects. 8-OH-Amoxapine is comparable to the parent compound's Norepinephrine reuptake blocking properties, however it is extremely potent on serotonin reuptake blockade. It has been demonstrated that 7-OH-Amoxapine has significant dopamine receptor blockade in laboratory animals and as such its dopamine (DA) blocking and neuroleptic activity in humans should be studied. We have recent data in amoxapine treated humans which demonstrates potent DA blocking effects in some patients and negligible effects in others. Plasma samples were obtained on thirty (30) patients maintained on amoxapine therapy for at least three (3) weeks. Plasma was assayed for 8-OH-Amoxapine by gas liquid chromatography and for dopamine receptor blocking activity by a Radio Receptor Assay (RRA). Significant dopamine blocking activity was noted by RRA in samples independent of plasma 8-OH-Amoxapine level. 7-OH-Amoxapine concentrations correlate with dopamine blocking activity by RRA. High concentrations of 7-OH-Amoxapine and RRA activity may be correlated with akathisia, E.P.S., galactorrhea, antipsychotic efficacy. Implications of these data, RRA level versus side effect ratings will be discussed. Occasional patients who are administered this compound on a chronic basis form high amounts of 7-OH-Amoxapine and who have high amounts of DA blocking activity may be at risk for tardive dyskinesia.

155.8 THE EFFECTS OF TRIMETHYLTIN ON DOPAMINERGIC AND SEROTONERGIC FUNCTION OF THE CENTRAL NERVOUS SYSTEM. D.L. DeHaven, T.J. Walsh* and R.B. Mailman. Biol. Sci. Res. Ctr., Univ. North Carolina Sch. Med., Chapel Hill, NC 27514 and Neurotoxicology Div., HERL, US EPA, Research Triangle Park, NC 27711

Trimethyltin (TMT) is a neurotoxic organometal which produces selective lesions in the limbic system, in particular the pyramidal cell fields of the hippocampus (Brown et al., Am. J. Pathol., 97:59, 1979). Acute administration of this compound also produces such behavioral effects as increased locomotor activity, hyperreactivity, and memory deficits in a variety of paradigms (Walsh et al., Neurobehav. Toxicol. Teratol., 4:177, 1982). The present studies provided neurochemical data relevant to those behavioral and pathological changes. Functional changes in dopamine (DA) and serotonin (5-HT) systems were determined by measuring the effects of TMT on concentrations of DA, 5-HT and their metabolites in brain. Male Long-Evans hooded rats (ca. 120 days old) were administered TMT hydroxide (3 or 7 mg/kg, IG) or vehicle (0.9% saline) and sacrificed for neurochemical analysis 7 days later. From each brain, the striata, olfactory tubercles, nucleus accumbens, and hippocampus were dissected and analyzed for dopamine, serotonin and their acidic metabolites, DOPAC, HVA and 5-HIAA by HPLC with EC detection (Kilts et al., J. Chromatog., 225:347, 1981). In some animals Scatchard analyses of the binding of ^{3}H-spiperone to individual striata were also performed using (+)-butaclamol to assess non-specific binding. The results of these experiments revealed that TMT had no effects on the affinity or number of ^{3}H-spiperone receptors in the striatum and did not alter the concentration of DA or its metabolites in any region examined. Although serotonin concentrations were not significantly altered, both 5-HIAA concentrations and the 5-HIAA/5-HT ratio were increased. Since the ratio of 5-HIAA/5-HT is an estimate of serotonin turnover, this suggests that increases in 5-HT turnover result from TMT treatment of adult rats. This effect of TMT on serotonergic function suggests that this neurotransmitter may be involved in the behavioral sequelae resulting from TMT-induced neurotoxicity. (Supported in part by NIH Grants ES-01104 and HD-03110 and EPA C R809644010).

155.9 OPEN TRIAL OF CLONIDINE IN DYSTONIA MUSCULORUM DEFORMANS. Donald Kay Riker, Howard Hurtig*, C. Raymond Lake, Paul Copeland*, and Robert Roth. Depts. Pharmac. & Psych., Yale Univ. Sch. Med., New Haven, CT; Depts. Neurol. & Medicine, Hosp. Univ. Penn., Phila., PA; Dept. Psych., Unfrm. Serv. Univ. Health Sci., Bethesda, MD.

Dystonia musculorum deformans (DMD) is an inherited movement disorder which provokes powerful movements and postures of limbs, neck, or trunk. Its etiology and progression are uncertain. Pathology and therapeutics have failed to suggest a mechanism or treatment. High levels of plasma dopamine-beta-hydroxylase and norepinephrine (NE) have been reported in DMD (New Eng J Med 288:284,1973; Adv Neurol 14:307,1976). To support these data we assayed NE levels in plasma and CSF, and assessed the efficacy of clonidine (C), a drug known to reduce NE metabolism.

Non-Jewish, sporadic patients were admitted to the Clinical Research Center at H.U.P. Five patients had rostral-segmental dystonia with torticollis. Four patients exhibited generalized dystonia (onset before 21). Diet was devoid of amines and stimulants. Dystonia was rated daily (0-5) in each body part. Blood was drawn through in-dwelling lines at 0800hrs in supine and standing position (2 hours after C). Lumbar puncture was performed the morning after each admission; patients later began C (50or100ug tid p.o.). After 3-11 days patients returned home on this dose (10-17days). If readmitted C was raised daily (max=1.5mg/day). Plasma/CSF NE, 3-methoxy-4-hydroxyphenethylene glycol (MHPG), and homovanillic acid (HVA) were assayed.

Patients presented with plasma NE values (Supine=550-1837; Stand=1086-5094pg/ml) above +1SD and up to 9-fold the normal mean (Supine:292+1SD=430; +2SD=568; Stand:538+1SD=830; +2SD=1122pg/ml). CSF NE values (465-921pg/ml) were above +1SD and up to 3.5-fold the normal mean (251+1SD=414;+2SD=577pg/ml). Plasma MHPG (1.95-5.67ng/ml) was within ±2SD's of normal mean (3.44±1.54ng/ml); most CSF MHPG values (5.54-11.76ng/ml) were below the normal mean (10.93±2.71ng/ml). Except for one hypertensive, no dysautonomia was noted. C reduced plasma & CSF NE to within, or below, normal limits dependent on dose. Plasma & CSF MHPG were weakly responsive; plasma HVA reacted transiently to dose shifts. However, C did not improve generalized dystonia. Segmental patients claimed transient worsening or improvement at low-doses. Simple torticollis in one patient did resolve.

In summary: 1) increased central (CSF) and peripheral (plasma) NE is a sign of DMD; 2) reduction of NE hypermetabolism does not reduce generalized dystonia. Reducing NE metabolism is likely to benefit only if increased metabolism were responsible for disorder, not if it is compensatory.--Supported by the Dystonia Medical Research Foundation, Beverly Hills, CA

155.10 EFFECTS OF ACUTE AND CHRONIC LITHIUM TREATMENT ON SEROTONIN UPTAKE IN BLOOD PLATELETS OF MANIC-DEPRESSIVE PATIENTS. R. C. Arora, P. J. Goodnick* and H. Y. Meltzer. Ill. State Psychiatric Institute and the Department of Psychiatry, Univ. of Chicago Pritzker School of Medicine, Chicago, IL. 60637.

A decrease in the Vmax (a measure of the number of uptake sites) of serotonin (5-HT) uptake in the blood platelets of patients with bipolar or unipolar depressive disorders has been reported by many investigators. This decrease is present during the acute phase as well as after recovery and appears to be a marker for vulnerability to develop depression or mania. While most first generation antidepressant drugs other than monoamine oxidase inhibitors decrease 5-HT uptake, the effect of acute or chronic treatment with lithium carbonate (Li) on human platelet, as on rodent brain 5-HT uptake, has been controversial with some studies reporting no effect and others significant decreases or increase.

Platelet 5-HT uptake was studied in patients with affective disorders at the onset of an acute episode, while on placebo, after 2-4 weeks of Li treatment and again after 1 year or more of Li treatment. The method used has been described earlier (Arora, R.C. and Meltzer, H.Y., Clin. Chim. Acta., 112:225, 1981). Unmedicated bipolar patients had significantly decreased Vmax (9.4 ± S.D. 2.0 pmoles/10^7 platelets/min. in 21 bipolar patients vs 11.9 ± S.D. 2.5 pmoles/10^7 platelets/min in 37 normal controls (p .005). The mean Vmax decreased further after 2-4 weeks of Li treatment in 14 manic patients (from 9.52 ± S.D. 2.0 pmoles/10^7 platelets/min to 7.44 ± S.D. 1.9 pmoles/10^7 platelets/min, $p < .005$). Vmax decreased in 11 of 14 manic patients and the mean decrease was 33% (range 12-50%). Six of these patients were restudied after 12 or more months of the continuous Li treatment. In 3 of these 6 patients, Vmax increased by 100% or more. A total of 14 patients treated with Li for more than one year, were also studied. Vmax was 17.00 ± S.D. 6.43 pmoles/10^7 platelets/min which was significantly greater than the Vmax of unmedicated bipolar patients or normal controls. Thus, our results indicate that Li produces time dependent changes in the Vmax in platelet uptake in most, but not all patients. If this also occurred in brain, it could be related to the sometimes rapid onset antimanic effects of Li treatment and the more slowly developing prophylactic effects of Li treatment on recurrent unipolar or bipolar disorders (Supported in part by USPHS MH 30059 and MH 25116).

155.11 AUTORADIOGRAPHS OF PARKINSONIAN VS. NORMAL HUMAN STRIATUM SUGGEST THAT HUMAN NIGROSTRIATAL DOPAMINE TERMINALS BEAR MANY TYPES OF BRAIN RECEPTORS. A. Larsen*, D. Calne, R. Quirion, M. Herkenham and C.B. Pert. National Institute of Mental Health and National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, MD 20205.

It has been elegantly demonstrated that Parkinson's Disease is associated with the degeneration of dopaminergic cell bodies in the substantia nigra and dopamine loss from the terminals of the striatal projections (Bernheimer et al., 1973; J. Neurolog. Sci. 20:415-455). A careful study of the cellular elements of normal vs. parkinsonian and male vs. female striata has failed to demonstrate any significant differences (Bottcher, Acta Neurologica Scand. 1975, 52, suppl 62, 1-87).

Over the last two years, we have subjected the striata of 5 parkinsonian patients and 6 non-parkinsonian patients to a procedure which autoradiographically visualizes brain receptors (Herkenham and Pert, J Neurosci., in press). We have experienced the usual difficulties in finding control and unequivocally diagnosed diseased brains which are well-matched for age, time elapsed from death to autopsy and all steps of subsequent processing.

Recently, we (A.L.) had the opportunity to obtain at autopsy a striatum from our 60-year-old NIH female patient who died from a pulmonary thromboembolism after suffering from Parkinson's Disease unresponsive to L-DOPA for four years, having reached the most severe stage (Hoehn and Yahr V) of extreme rigidity at the time of her death, which occurred 18 hours before autopsy. A striatum from a 68 year old male NIH patient who died from sepsis from aplastic anemia almost 23 hours before autopsy provided a well-matched control, biased, if at all, against the direction of the results we expected from previously examined, less well-matched material. Striata were frozen on dry ice immediately after removal and maintained at -70°C until cryostat sectioning. All storage, cutting, and subsequent receptor labeling and analysis was conducted in parallel.

Our previous impressions were confirmed in this well-controlled study: the parkinsonian striatum contained substantial (26-58%) losses in ^{3}H- diazepam, naloxone, spiperone, D-Ala-DLeu enkephalin and neurotensin binding, labeled under well-characterized receptor-related conditions. Cortically localized beta-adrenergic receptor levels were not significantly different. Visualization of these patterns of receptor loss will be presented. Like rat (e.g., see Bowen et al. and Quirion et al, this meeting), human nigrostriatal projections seem richly encrusted with numerous brain receptors offering great potential for endogenous modulatory control and exogenous therapeutic manipulation.

155.12 IMMUNOREACTIVE VASOACTIVE INTESTINAL PEPTIDE IN CEREBROSPINAL FLUID. N.S. Sharpless, L.J. Thal*, M.J. Perlow, K. Tabaddor*, J. Waltz*, K. Shapiro*, J. Engel, Jr. and P.H. Crandall*. Albert Einstein College of Medicine, Bronx, NY 10461

Vasoactive intestinal peptide (VIP) is localized in the synaptosomal fraction of mammalian central nervous tissues suggesting a role for VIP in neurotransmission. To gain insight into the relationship between VIP and neurological disease, we used a sensitive radioimmunoassay to measure VIP concentrations in lumbar and ventricular cerebrospinal fluid (CSF) from patients with various neurological disorders and in 2 hr aliquots of cisternal fluid removed continuously from rhesus monkeys. Aliquots of CSF (400 µl) were incubated 24 hr at 4° C with 300 µl of phosphate buffer (0.06 M, pH 7) containing albumin (1.5%), aprotinin (2000 KIU/ml), and rabbit antiserum to VIP (donated by G. Nilaver). ^{125}I-VIP (100 µl) was then added and the mixture was incubated at 4° C for 3 more days. Antibody-bound and free ^{125}I-VIP were separated with 10 mg of dextran coated charcoal. A standard curve was generated by analyzing synthetic porcine VIP (0.5 to 100 fmol) in artificial CSF. The limit of sensitivity of the assay was 2.5 fmol per ml of CSF. Dilution curves of VIP-like immunoreactivity in human CSF and of synthetic porcine VIP added to artificial or human CSF were parallel. VIP in concentrated pools of human ventricular CSF and of monkey cisternal CSF co-eluted with synthetic porcine VIP on a column of Sephadex G-25. In the monkeys, a circadian pattern of CSF VIP concentration was observed in 2 of 3 animals, with highest levels occurring at night and lowest during the day. VIP levels were similar in the first and last 6 ml of a total of 24 ml of CSF withdrawn from the lumbar region of 3 Alzheimer patients and in samples of ventricular and lumbar CSF from the same seizure patients, indicating that there is no significant craniocaudal CSF VIP concentration gradient. Nevertheless, mean VIP levels were slightly higher in lumbar CSF from patients with multiple sclerosis (6.5 fmol/ml;N=12), Alzheimer's disease (8.7 fmol/ml;N=8), epilepsy (8.9 fmol/ml;N=10), and a mixed group of patients with various disorders (9.5 fmol/ml;N=29) than in ventricular CSF from patients with multiple sclerosis (4.4 fmol/ml;N=11), dystonia (5.6 fmol/ml;N=13), Parkinson's disease (5.4 fmol/ml; N=15), or epilepsy (4.5 fmol/ml;N=8). Highest ventricular fluid VIP concentrations were seen in hydrocephalic children before shunt insertion and lowest in patients with acute cerebral trauma. The mean concentration of VIP was significantly decreased in lumbar CSF from patients undergoing aneurysm surgery. The results suggest that there are differences in CSF VIP concentrations in different patient populations which may provide insight into the underlying nature of their disorder. (Supported by NIH grant NS-09649 and by a grant from the Dystonia Medical Research Foundation).

155.13 GROWTH HORMONE RESPONSES IN UNTREATED HUNTINGTON'S DISEASE PATIENTS: EVIDENCE FOR DIFFERENT SUBGROUP POPULATIONS. A. Martinez-Campos+, P. Giovannini+, G. Scigliano+, A. Novelli+, E.A. Parati, T. Caraceni+ and E.E. Müller+. Department of Pharmacology, University of Milan, Istituto Neurologico "C. Besta", Milan, Italy.

Evidence has been provided for the existence of an altered growth hormone (GH) responsiveness to dopaminergic (DA) stimuli in patients with Huntington's Disease (HD), though contradictory findings have been reported in the literature. Previously, we demonstrated GH hyperresponsiveness to the DA agonists bromocriptine, apomorphine and l-dopa in most of 30 HD patients washed out of therapy (Müller et al., Adv. Neurol. 23: 319, 1979). These findings were at variance with reports by others of GH hypo-normo responsiveness to the same drugs in HD patients clinically undistinguishable from ours.

In this study we decided to re-evaluate this problem in a group of 12 untreated HD patients with the use of bromocriptine (Parlodel, Sandoz, 2.5 mg orally) and/or lisuride (Dopergin, Schering, 0.2 mg orally), an ergoline derivative with potent DA-mimetic properties. Baseline GH levels were not significantly different in patients or sex-and-age-matched controls. Of the 12 patients investigated, lisuride induced a significantly lower GH response than in controls in 6, a normal GH response in 3 and a significantly greater GH response in 3 subjects. Bromocriptine, administered to 7 of the same subjects elicited GH responses qualitatively superimposable to those induced by lisuride (GH hyporesponsiveness in 4, normoresponsiveness in 2 and hyperresponsiveness in 1 subject). In addition, application of a potent and long-acting met-enkephalin analogue (FK 33-824, Sandoz, 0.5 mg/ iv), which to increase GH secretion requires, at least partly, the integrity of hypothalamic DA function, reiterated the findings obtained with the DA agonists. Three of the bromocriptine and lisuride hyporespnders were FK 33-824 non-responders, while 3 bromocriptine and lisuride normoresponders had a proper GH response to the opioid peptide. In individual patients GH responsiveness to DA agonists was unrelated to the clinical parameters considered (age, dementia, duration and/or severity of the disease, hyperkinesia, emaciation).

These data obtained in untreated HD patients demonstrate that: 1) the pattern of GH responsiveness to DA-mediated stimuli does not accur at random but is reproducible and reflects in many subjects the existence of a disturbed DA control of GH secretion; 2) HD subjects, though clinically indistinguishable one from another can be divided into subgroups according to a neuroendocrine index.

155.14 DECREASED BRAIN DOPAMINE SYNTHESIS RATE IN STREPTOZOTOCIN-DIABETIC RATS. C. D. Himmel* and M. E. Trulson (SPON: R. Stillman). Lab. for Neurobiology, Univ. of Texas at Dallas, Richardson, TX 75080.

Glucose administration completely suppresses the discharge rate of dopamine-containing substantia nigra neurons in rats (C. F. Saller and L. A. Chiodo, Science, 210, 1980, 1269), but it is not known whether hyperglycemia alters central dopamine metabolism. Accordingly, we investigated the rate of dopamine synthesis in diabetic rats with blood glucose levels 5-6 times higher than those in normal rats. Female Sprague-Dawley rats were made diabetic by injections of streptozotocin (75 mg/kg, i.p.) dissolved in citrate buffer (pH 4.5), while control rats received injections of buffer only. Diabetes was verified by glucosuria and hyperglycemia. Rats were maintained on ad libidum food and water for 4-6 weeks after induction of diabetes, and then killed by decapitation for neurochemical analysis. In insulin replacement studies, diabetic rats (4-6 weeks after streptozotocin) were administered protamine zinc insulin (4 IU/kg, s.c.) every 12 h for 10 consecutive days prior to the time of assay. Control and diabetic rats were administered a decarboxylase inhibitor (RO4-4602, 800 mg/kg, i.p.) and subgroups of rats were killed for assay of L-3,4-dihydroxyphenylalanine (DOPA) in the corpus striatum and limbic forebrain (nucleus accumbens, olfactory tubercle, and frontal cortex) 0, 30 or 60 min later. The tissues from 3 rats were pooled for all assays, and DOPA was measured spectrofluorimetrically after isolation by ion-exchange chromatography. DOPA accumulation was significantly decreased by 43.2% in the corpus striatum (2.25 $\pm$ 0.09 nmol/g/h) as compared to vehicle control (3.96 $\pm$ 0.11 nmol/g/h) and by 30.4% in the limbic forebrain (2.24 $\pm$ 0.10 vs 3.22 $\pm$ 0.12 nmol/g/h in the vehicle control). Mean blood glucose levels for all diabetic rats was 594.1 $\pm$ 13.3 mg% as compared to 99.8 $\pm$ 4.4 mg% for vehicle controls. Insulin replacement therapy restored both DOPA accumulation and blood glucose levels to normal. Receptor binding studies revealed that the B_{max} of ^{3}H-spiroperidol binding in the corpus striatum was significantly increased by 34.6% in diabetic rats (28.8 $\pm$ 0.9 pmol/g) as compared to vehicle controls (21.4 $\pm$ 1.2 pmol/g). These data confirm a previous report (Lozovsky, et al., Science, 214, 1981, 1031). We also observed a significant increase in ^{3}H-spiroperidol binding of 32.0% in the limbic forebrain (16.1 $\pm$ 0.9 pmol/g) as compared to vehicle control (12.2 $\pm$ 1.1 pmol/g). These changes in receptor binding were also restored to normal by insulin replacement therapy on the treatment regimen described above. These data demonstrate that the in vivo rate of dopamine synthesis is reduced in untreated diabetic rats. Furthermore, these changes are apparent in both the nigral-striatal and meso-limbic dopamine systems. The increased dopamine receptor binding may be a compensatory mechanism to offset the decreased dopamine synthesis rate.

155.15 ENZYMATIC DECONJUGATION OF CATECHOLAMINES (CA) IN HUMAN AND RAT PLASMA AND RED BLOOD CELLS (RBC). S. Yoneda* N. Alexander, N. Vlachakis* (SPON: B. Newman) Univ. So. Cal. Sch. of Med. Los Angeles, CA 90033

The evidence that conjugated CA may be converted to free CA (Buu & Kuchel, Life Sci. 24:783, 1979) and that they represent a large proportion of the pool of released CA indicates the potential physiological significance of conjugated CA. We have developed a new enzymatic method for hydrolysis of conjugated CA. Our method hydrolyses both sulfate and glucuronide conjugation of CA completely and is applicable not only to plasma but also to RBC whose conjugated CA cannot be measured by acid hydrolysis (unpub. obs.). Furthermore, the method is more reliable, sensitive and simpler than acid hydrolysis or lyophilization, because it has better and more consistent recovery of internal standards, lower blanks and no added steps for hydrolysis. In our study the CA results obtained by enzymatic hydrolysis were compared to those of acid hydrolysis plus heating (95°C) with a radioenzymatic assay. Plasma total CA and conjugated CA are expressed in pg/ml and % (mean+SEM), respectively, and RBC values are shown in parenthesis:

HUMAN (n=6)		NOREPI (NE)	EPI (E)	DOPAMINE (DA)
Enzy.	Total	1733±145(866±88)	294±18(292±46)	4076±482(274±659)
Hydr.	Sulf.C.	67±1 (42±5)	85±3 (62±4)	99.2±0.3(100)
	Gluc. C.	6±1 (0)	3±1 (0)	0.5±0.1(0)
Acid	Total	1354±167	226±16	4490±529
Hydr.	C.	65±2	86±3	99.7±0.2
RAT (n=5)				
Enzy.	Total	563±40	119±21	1762±264
Hydr.	Sulf.C.	41±1	14±5	8.6±1.8
	Gluc. C.	8±3	27±2	90.6±1.7
Acid	Total	443±36	138±38	314±54
Hydr.	C.	33±7	40±10	95.3±2.2

In human plasma, CA are conjugated almost entirely with sulfate while in rat plasma, glucuronides are the main conjugates of E and DA but not NE. Acid hydrolysis of rat plasma is significantly less than by enzymatic hydrolysis (p 0.01), which may reflect high stability of glucuronide CA conjugation. In human RBC, free NE and E is higher than in plasma (Alexander et al., Life Sci. 29: 471, 1981) however, conjugated NE and E are lower in RBC than in plasma (NE, p 0.001; E, 0.1 0.05). The direct evidence presented here of RBC sulfate conjugates along with reported RBC sulfotransferase activity, indicates that RBC are a site of sulfate conjugation for CA.

156.1 ENDOGENOUS PROTEIN PHOSPHORYLATION IN CHICK AND RAT SYNAPTIC MEMBRANES. R.G. Sorensen* and J.A. Babitch. Chemistry Dept., Texas Christian Univ., Ft. Worth, TX 76129.

We have compared the cAMP-mediated and Ca^{2+}-mediated protein kinase (PK) activities endogenous to synaptic membranes prepared by an identical procedure from chick (avian) and rat (mammalian) brain. Both species showed similar responses towards the protein kinase effector molecules, cAMP and Ca^{2+}. Half-maximal stimulation ($K_{0.5}$) of cAMP-PK activity occurred at 0.4-0.8 µM cAMP. The $K_{0.5}$ of Ca^{2+}, CaM-PK activity occurred at 1-2 µM Ca^{2+}_{free} both in the absence or presence of CaM added to the reaction mixture suggesting that the CaM present in these membranes was able to modulate Ca^{2+}, CaM-PK activity. After EGTA-treatment to remove the endogenous CaM, no significant response towards Ca^{2+} was measured in the absence of CaM and the $K_{0.5}$ was increased to 15 µM Ca^{2+}_{free} in the presence of CaM. There was a difference in the maximal levels of kinase activity in these membranes with chick membranes containing 57% less cAMP-PK activity, but 65% more Ca^{2+}, CaM-PK activity than the rat membranes as measured by liquid scintillation counting of ^{32}P in gel slices cut from the polyacrylamide gels used to separate the labelled membrane polypeptides. Similar results were determined when either low (5 µM) or high (5.8 mM) concentrations of ATP were added to the reaction mixtures.

Besides certain species differences in the molecular weights of the resulting phosphoproteins, we observed several major differences with respect to the absence or presence of some of the phosphoproteins. Chick membranes lack the cAMP-requiring, microtubule-associated phosphoprotein, MAP_2, and one of the 2 neuron-specific, cAMP-requiring and Ca^{2+}, CaM-requiring phosphoproteins (Protein Ib, although Protein Ia is present), and the Ca^{2+}-requiring, CaM-independent, ACTH-sensitive phosphoprotein, B50.

Several effector substances were tested for their ability to modulate Ca^{2+}, CaM-PK activity of both membrane preparations. The phenothiazines, TFP, FLU and CPZ, all inhibited this activity and the inhibition appeared to be specific towards CaM because the phenothiazine analogue, CPZ-sulfoxide, had no effect on Ca^{2+}, CaM-PK activity. Also found to inhibit Ca^{2+}, CaM-PK activity were dibucaine and ACTH.
(Supported by Research Grant NS-12485-07 from NIH.)

156.2 ANALYSIS OF GLUCOCORTICOID AND CYCLIC AMP EFFECTS ON PROTEIN EXPRESSION AND PHOSPHORYLATION IN HIPPOCAMPAL SLICES USING TWO-DIMENSIONAL GEL ELECTROPHORESIS. A.M. Etgen and E.T. Browning. Dept. Biol. Sci., Rutgers Univ., New Brunswick, NJ 08903 and Dept. Pharmacol., Rutgers Med. School, Piscataway, NJ 08854.

Steroid hormones and cyclic nucleotides regulate many intracellular events in the nervous system, including protein expression and phosphorylation. Since the hippocampus is the primary CNS target for glucocorticoids and since the electrophysiology of hippocampal slices has been extensively investigated, we utilized 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE) to examine the effects of corticosterone (CORT) and cyclic AMP (cAMP) on protein expression and phosphorylation in rat hippocampal slices. Analysis of Coomassie- and silver-stained gels from slices indicated that the protein patterns were indistinguishable for slices derived from left vs right and dorsal vs ventral hippocampus. Exp.1 examined the effects of cAMP and CORT on protein phosphorylation. Transverse hippocampal slices were prepared from gonadectomized, adrenalectomized adult female Sprague-Dawley rats which had (+CORT) or had not (-CORT) received steroid replacement via 5 mm Silastic implants (cholesterol:CORT, 1:1). Single slices (+ and -CORT) were incubated in low phosphate (0.2 mM) Yamamoto's medium for 3 hr with ^{32}Pi. Slices from +CORT animals were incubated with 10 nM CORT. cAMP synthesis was stimulated during the final 20 min by 20 uM forskolin, which directly activates most, if not all adenylate cyclases. cAMP levels in slices were measured using a modified Gilman binding assay. In the absence of forskolin, all slices had similar cAMP levels regardless of steroid treatment (2.0-2.5 pmole/mg protein). Forskolin elevated cAMP levels in both + and - CORT slices by 40- to 60-fold. Analysis of the autoradiograms of 2-D gels from ^{32}P-labeled slices revealed that forskolin also increased the phosphorylation of two spots (55K & 62K; pI's near 7) in both + and - CORT slices. CORT decreased the phosphorylation of glial fibrillary acidic protein (GFA). Forskolin stimulated GFA phosphorylation in CORT treated slices. Exp.2 investigated the effects of CORT on protein expression in hippocampal slices prepared from + and -CORT animals and maintained in standard Yamamoto's medium. After a 1 hr equilibration, ^{35}S-methionine was added to the slices, and the incubation was continued for 2.5 hr. Proteins were fractionated by 2-D PAGE. Initial results indicate that CORT may regulate the incorporation of ^{35}S-methionine into several proteins, including putative vimentin, GFA and several other unidentified proteins. (Supported by Grant BRSG PHS RR7058 and NSF Grant BNS 81-10564.)

156.3 GTP-PREFERRING PROTEIN PHOSPHORYLATION SYSTEMS IN BRAIN MEMBRANES: POSSIBLE ROLE IN ADENYLATE CYCLASE REGULATION. Y.H. Ehrlich, S.R. Whittemore*, R. Lambert*, J. Ellis*, S.G. Graber*, and R.H. Lenox. Depts. Psychiatry, Biochemistry and Physiology/Biophysics, Univ. of Vermont College of Medicine, Burlington, VT 05405.

Previous studies in our laboratory have demonstrated that when brain membranes are preincubated under dephosphorylating conditions, adenylate cyclase (AC) activity is inhibited. Conversely, preincubation under phosphorylating conditions(with ATP and $MgCl_2$) stimulated adenylate cyclase activity, whereas thiophosphorylation with ATP-γ-S during preincubation resulted in irreversible activation of AC. Assays carried out with ATP-γ-[^{35}S] have identified a protein band with apparent MW of 54,000 daltons (54K) whose phosphorylation correlated significantly with AC activation (Whittemore et al., Neurochem. Res. 6:775, 1981; Neurosci. Abst. 7:920, 1981, and manuscript submitted for publication). In the present studies, we have examined whether this mode of regulation may involve endogenous protein phosphorylation systems that prefer to utilize GTP, rather than ATP, as a phosphate donor.

Preparations containing synaptic membranes (shocked P_2) from the neostriatum or cerebral cortex of decapitated rats were incubated with [γ-^{32}P]GTP or [γ-^{32}P]ATP of equal specific activity and then subjected to SDS-slab gel electrophoresis and autoradiography to identify the endogenously phosphorylated proteins. In reactions carried out with 1µM $GT^{32}P$ and 10 mM $MgCl_2$, a major substrate was a phosphoprotein band with MW of 54K and a minor band had a MW of 33K. Substituting $MnCl_2$ for $MgCl_2$ resulted in a marked increase in the phosphorylation of these proteins. In fact, in the presence of Mn++, the 54K and 33K protein substrates were phosphorylated by $GT^{32}P$ but not by $AT^{32}P$. Further characterization of the phosphorylation of the 54K and 33K substrates by GTP revealed that this activity is inhibited by cyclic AMP, independent of Ca++-ions and is regulated by a mechanism that may involve the phosphorylation of a 47K protein by ATP. Triton-X-100 extract of brain membranes containing the catalytic and regulatory components of AC was found to be highly enriched in endogenous activity which phosphorylates the 54K band with Mn·GTP, but not with ATP. Finally, preincubation of membranes with GTP and divalent cations caused activation of AC which withstood sedimentation and washings. These findings provide the means for direct testing of the possibility that a 54K substrate of a GTP-utilizing protein kinase plays a role in AC regulation, and by extention--in neural receptor adaptation (Adv. Exptl. Med. Biol. 116:75, 1979 and Progress in Brain Res., 1982). Supported in part by grants DA02747 and MH35735.

156.4 CALCIUM/CALMODULIN-DEPENDENT PROTEIN PHOSPHORYLATION IN APLYSIA NEURONS. S.A. DeRiemer, L.K. Kaczmarek and P. Greengard. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

A rise in intracellular cAMP triggers a long lasting afterdischarge in the Bag Cell neurons of Aplysia. Changes in the phosphorylation state of two specific Bag Cell proteins, BC I (M_r 33,000) and BC II (M_r 21,000), have been observed during an afterdischarge. These two proteins are also substrates for a cAMP-dependent protein kinase (cAMP-PK) in broken cell preparations (Jennings et al., J. Neurosci. 2(2):158-168, 1982). Since there is a large inward calcium component to the action potentials of the Bag Cell afterdischarge, and since calcium-dependent protein phosphorylation has been shown to mediate some of the second messenger actions of calcium in other systems, we have begun a study of calcium-dependent protein phosphorylation in Aplysia.

We have examined endogenous protein phosphorylation in homogenates of isolated Bag Cell clusters, as well as in homogenates of the remainder of the CNS of Aplysia, using sodium dodecyl sulfate polyacrylamide gel electrophoresis to analyze the pattern of phosphorylated substrates. Results with the two preparations were similar except in the case of the Bag Cell specific protein, BC II. In addition to the cAMP-PK previously described, we have found a protein kinase activity dependent on calcium plus calmodulin (Ca/CaM-PK). Three classes of endogenous substrates were observed: 1. Proteins that are substrates for cAMP-PK but not for Ca/CaM-PK such as BC I. 2. Proteins that are substrates for Ca/CaM-PK but not for cAMP-PK. The major substrate in this class is an M_r 51,000 protein which by peptide mapping resembles a Ca/CaM-PK substrate from mammalian brain. 3. Substrates for both cAMP-PK and Ca/CaM-PK (M_r 80,000 and the previously described Bag Cell specific BC II).

Ca/CaM-PK activity was inhibited by drugs which block other calmodulin dependent processes. The drugs tested, in decreasing order of potency, were R24571 > trifluoperazine > chlorpromazine > W7. The ID_{50} of trifluoperazine was 6 µM. The physiological effects of these drugs on the Bag Cell afterdischarge are under investigation.

These results suggest that protein phosphorylation may mediate some of the actions of calcium in Aplysia neurons in general and during the Bag Cell afterdischarge in particular.

156.5 CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE IN INTERNALLY PERFUSED SNAIL NEURONS IS ENHANCED BY PROTEIN PHOSPHORYLATION. Irwin B. Levitan, Jacques E. de Peyer*, Armand B. Cachelin* and Harald Reuter*. Friedrich Miescher-Institut, Basel, Switzerland and Dept. of Pharmacology, Univ. of Berne, Switzerland.

Depolarizing voltage steps induce inward and outward currents in voltage clamped, internally perfused neurons from the snail Helix roseneri. Addition of 0.01 to 1 μM of the catalytic subunit of cyclic AMP-dependent protein kinase to the internal perfusing medium results in an increase in the net outward current, with no apparent effect on the inward current. In addition, the catalytic subunit causes an increase in the rate of activation of the outward current. DTNB-inactivated catalytic subunit (1 μM) is without effect, indicating that the change in outward current results from protein phosphorylation rather than simply from perfusion of protein per se. Decreasing the external calcium concentration from 10 to 1 mM eliminates the effect of catalytic subunit, suggesting that calcium plays an important role in this response. This suggestion is supported by the finding that the enhancement and increase in rate of activation of outward current can be mimicked by increasing the calcium concentration in the internal perfusing medium. Furthermore, when 10 mM EGTA is added to the internal perfusing medium to completely suppress the calcium-activated potassium conductance, there is no effect of catalytic subunit on the remaining component of outward current (the delayed rectifying current). The results are consistent with the hypothesis that cyclic AMP-dependent protein phosphorylation can regulate the calcium-activated potassium conductance in these cells.

156.6 INDEPENDENCE OF CHOLINERGIC- AND HISTAMINE-STIMULATED INCREASES IN PHOSPHATIDYLINOSITOL TURNOVER IN CLONED CELL LINES. Neri M. Cohen* and William L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

The character of the histamine-mediated increase in phosphatidylinositol (PI) turnover was investigated in neuroblastoma N1E-115 cloned cells. This study was greatly aided by the availability of a one-dimensional thin-layer chromatography (TLC) solvent system (acetone:butanol:acetic acid:water :: 5:5:1:1) (Cohen et al., J. Neurochem., in press) for the rapid and consistent separation of PI from other major phospholipids. Histamine stimulation caused as much as a 430% increase in the incorporation of 32P into PI, with the level of stimulation being dependent on the age and maturity of the cultures. Denser and more mature cells showed a significantly lower level of stimulation than immature cells. 10 -7 M. pyrilamine completely blocked the response to histamine stimulation and 10 -7 M. metiamide had no effect on the response, indicating that the histamine PI response is mediated by the H1 and not the H2 receptor. Removal of extracellular Ca++ by the addition of excess ethyleneglycoltetraacetic acid (EGTA) had no effect on the ability of histamine to stimulate the increased turnover of PI. The time course for increased incorporation of 32P into PI was similar to that observed previously for the cholinergic-mediated response, with stimulated rates of uptake significantly decreased by 30 minutes and no different from control rates by 40 minutes. Dual stimulation of both the cholinergic and histamine systems simultaneously produced an additive effect on the increased turnover of PI. Desensitization of the cholinergic-mediated stimulation of increased turnover had no effect on the ability of histamine to mediate a second stimulation of PI turnover. The notion of discrete functional units within the membrane, each containing a specific receptor and the machinery for the physiological reactions mediated by that receptor, is supported by these results.

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156.7 TEMPERATURE DEPENDENT CONFORMERS OF CALMODULIN. P. Gangola* and H. C. Pant (SPON: H. Gainer). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism. Rockville, MD 20852.

Conformational changes occur when calcium binds to calmodulin (CaM) which has been found to regulate a large number of fundamental cellular processes. We report here temperature induced conformational changes in calmodulin using the intrinsic tyrosine fluorescence. Calcium bound CaM undergoes a reversible conformational change in the temperature range 22-30°C. The transition temperature depends upon the concentration of calcium bound to CaM. In fully Ca^{2+}-bound CaM the transition occurs at slightly higher temperature than when the Ca^{2+} concentration is lower and the CaM is not fully bound. The apo-form of protein does not show this transition in the fluorescence intensity.

CaM has four binding sites or domains. Domains I and II contain no tyrosine residues while domains III and IV each have one tyrosine. A difference in the sequence of Ca^{2+} filling of these four sites is observed for the two temperature dependent conformers. For the low temperature conformer (which exists from 5-20°C), Ca^{2+} first fills one of the non-fluorescent domains (I or II); the next two sites to be filled are the fluorescent domains (III and IV), while the last site filled is the remaining, unfilled non-fluorescent site. For the conformer existing above 30°C, both of the non-fluorescent sites (I and II) are filled first followed by the fluorescent sites (III and IV). This temperature dependent transition is also affected by pH.

The CaM-Ca complex exists as two distinct conformers depending upon the temperature of the system with the calcium filling sequence being different for these two conformers. Furthermore, our results indicate that temperature induced conformational changes in CaM increase the Ca^{2+} binding affinity of one of the non-fluorescent sites (I or II).

156.8 [^{3}H]NITRENDIPINE BINDING IS REGULATED BY INORGANIC AND ORGANIC CALCIUM BLOCKERS: DIFFERENCES BETWEEN CENTRAL AND PERIPHERAL CALCIUM CHANNELS. K.M.M. Murphy, R. Gould* and S.H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Recent advances in the pharmacology of calcium and calcium blockers have allowed the identification of distinct sites of actions of various classes of inorganic and organic calcium antagonists. Lanthanum and cobalt are potent and selective inorganic calcium entry blockers. Nifedipine, verapamil and diltiazem are examples of three structurally different classes of organic calcium channel blockers.

[^{3}H]Nitrendipine labels high affinity sites in brain, myocardium, smooth muscle, and skeletal muscle which appear to represent the pharmacologically relevant site of action for the dihydropyridine calcium channel blockers. Thus in brain, heart and skeletal muscle, the potency of the various dihydropyridines at [^{3}H]nitrendipine sites parallels their physiologic potency. Other classes of calcium antagonists appear to interact allosterically with this site.

In brain membranes, [^{3}H]nitrendipine binding is absolutely dependent upon calcium ions. The ionic calcium agonists strontium or barium can replace calcium in this regard. Lanthanum, cobalt or other inorganic calcium blockers inhibit the calcium stimulation of [^{3}H]nitrendipine binding. These ionic interactions occur at some site distinct from the dihydropyridine binding site.

Interactions with other classes of calcium antagonists are apparent. For example, verapamil but not diltiazem, can regulate [^{3}H]nitrendipine binding. Verapamil's potent inhibition of [^{3}H]nitrendipine binding is non-competitive and sensitive to ionic regulation, suggesting a site of action separate from those of the dihydropyridine calcium blockers.

[^{3}H]Nitrendipine labeled calcium channels in peripheral tissue differ in the sensitivity to ionic regulation. Removal of endogenous cations by EDTA is less effective in lowering [^{3}H]nitrendipine binding in heart and skeletal muscle than in brain. In addition, complete restoration of those sites by addition of calcium ions, as seen in brain, is not observed in these peripheral tissues. However, the binding sites do appear sensitive to the inorganic calcium antagonists, lanthanum and cobalt. In addition, verapamil and D-600 regulate binding in a manner similar to their action in central nervous tissues.

Differential regulation of [^{3}H]nitrendipine binding in various tissues by distinct classes of calcium blockers may provide a basis for selective pharmacologic intervention at calcium channels in different tissues.

156.9 IN VITRO AUTORADIOGRAPHIC LOCALIZATION OF CALCIUM CHANNELS IN RAT BRAIN USING [^{3}H]NITRENDIPINE. R.J. Gould*, K.M.M. Murphy, and S.H. Snyder. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Calcium entry into cells regulates a multiplicity of excitation-secretion coupling processes. Depolarization-induced calcium entry into nerve terminals through voltage-dependent channels may regulate neurotransmitter release.

The dihydropyridine calcium channel antagonist [^{3}H]nitrendipine labels sites in the brain and peripheral tissues which are related to the pharmacologically relevant site of action of these drugs. These sites are regulated differentially by the inorganic calcium agonists and antagonists, and interact allosterically with sites recognizing verapamil, a structurally different calcium channel blocker. In guinea pig and rat, there are high numbers of [^{3}H]nitrendipine binding sites in the brain, heart, and skeletal muscle with other tissues having lower levels.

Within the brain of both species, striking regional differences exist in the numbers of [^{3}H]nitrendipine sites, with the pattern of regional distribution similar for both species. The hippocampus shows the highest numbers of specific [^{3}H]nitrendipine binding sites. The striatum, cerebral cortex and olfactory bulb also demonstrate high levels while the cerebellum, thalamus and hypothalamus have approximately half the number of sites of these regions. The midbrain and brainstem show substantially reduced amounts of [^{3}H]nitrendipine labeled sites.

In vitro autoradiography has been employed to determine the localization of [^{3}H]nitrendipine binding sites at a finer anatomical resolution. The cerebral cortex, for example, demonstrates a diffuse distribution of [^{3}H]nitrendipine binding sites while the hippocampal formation shows localization to the stratum molecularis of the dentate gyrus and the stratum radiatum. Substantially lower levels are seen in the granular layer of the dentate gyrus and the pyramidal layer of the hippocampus. In the olfactory bulb, the [^{3}H]nitrendipine binding sites are clearly localized to the external plexiform layer with the glomeruli and mitral cell layers having markedly reduced numbers of sites.

The distinct regional distribution of [^{3}H]nitrendipine binding sites within the brain suggests the possibility of an endogenous neurohumour which may regulate calcium fluxes via nitrendipine binding sites. The identification of such a factor will be facilitated by in vitro [^{3}H]nitrendipine binding and a knowledge of the specific receptor localization.

156.10 THE INTERACTION OF CINNARIZINE AND RELATED ANALOGUES WITH RECEPTOR SITES FOR CALCIUM CHANNEL ANTAGONISTS. F.J. Ehlert*, W.R. Roeske* and H.I. Yamamura. (SPON: D.J. Jenden). Dept. of Pharmacol. Univ. of Arizona Health Sciences Center, Tucson, Arizona 85724.

The interaction of cinnarizine and related analogues with receptor sites for calcium channel antagonists was investigated in ligand-binding studies using the potent dihydropyridine, [^{3}H]nitrendipine. Initial studies indicated that [^{3}H]nitrendipine bound in a saturable manner to homogenates of the rat cerebral cortex, heart and longitudinal muscle of the ileum. The specific component of [^{3}H]nitrendipine binding was consistent with mass-action behavior and was characterized by a high affinity dissociation constant in the range of 0.1 - 0.3 nM. Several other potent dihydropyridines competitively inhibited the specific component of [^{3}H]nitrendipine binding with a potency in the 10^{-10} - 10^{-9} M range. Verapamil and its methoxy derivative, D600, also inhibited [^{3}H]nitrendipine binding but were somewhat less potent and caused an allosteric inhibition of binding. Cinnarizine, flunarizine and lidoflazine also inhibited [^{3}H]nitrendipine binding with IC_{50} values of approximately 10^{-6} M in both the heart and cerebral cortex of the rat. Analysis of the data suggested that the inhibition of [^{3}H]nitrendipine binding by cinnarizine was allosteric. Preliminary experiments suggest that cinnarizine, flunarizine and lidoflazine also cause a qualitatively similar inhibition of [^{3}H]nitrendipine binding in the longitudinal muscle of the rat ileum. The results of our binding experiments investigating several structural analogues of calcium channel antagonists suggest that [^{3}H]nitrendipine is a useful probe for characterizing binding sites for calcium channel antagonists, and perhaps, for detecting tissue differences in the nature of these sites.

156.11 CALCIUM ANTAGONIST BINDING AND CALCIUM UPTAKE INHIBITION IN A CLONAL CELL LINE. Lawrence Toll. Bio-Organic Chemistry Laboratory, Life Sciences Division, SRI International, Menlo Park, California 94025

The pharmacology of the voltage-dependent calcium channel has come under close scrutiny recently due to the labeling with tritium of the calcium antagonist nitrendipine. Using receptor binding techniques, several groups have determined binding characteristics of [^{3}H]nitrendipine and other dihydropyridines, as well as other classes of calcium antagonists. Binding has been studied in brain, heart, ileum and other organs. In all cases, receptor affinity correlates with pharmacological potencies in inhibiting smooth muscle contraction. I now report the binding of calcium antagonists and their inhibition of ^{45}Ca uptake in a phenochromacytoma clonal cell line.

The phenochromacytoma cell line, PC12, has been used as a model system for the study of storage and calcium-dependent release of acetylcholine and catecholamines. Properties of Na^+ and Ca^{++} fluxes have also been studied in this cell line. [^{3}H]-Nitrendipine binding to PC12 cell membranes is similar to binding in brain and other organs. Binding is saturable, with a K_D of about 1 nM. The receptor density is about 30 fmol per mg protein, or about 1,650 calcium channels per cell.

[^{3}H]Nitrendipine binding can be displaced by other dihydropyridine calcium antagonists with affinities between 1 and 5 nM. [^{3}H]Nitrendipine binding is also inhibited by compound D-600 (methoxyverapamil) which also is known to inhibit calcium uptake in smooth muscle and other tissue. The IC_{50} of D-600 is about 10 μM, however, maximal inhibition is not as great as with the dihydropyridine compounds.

Affinities of the calcium antagonists at the [^{3}H]nitrendipine binding site were compared with their abilities to inhibit the depolarization induced ^{45}Ca uptake into PC12 cells. Increasing potassium from 5 to 55 mM increases ^{45}Ca accumulation 5-fold. Uptake is rapid, being half maximal within 30 sec. Inhibition of potassium induced ^{45}Ca uptake by the calcium antagonists correlates very well with their inhibition of [^{3}H]nitrendipine binding. The dihydropyridine inhibitors have half-maximal inhibition in the range of 1-5 nM, while D-600 is about 5 μM. With 1.3 mM $CaCl_2$ in the incubation buffer, 1.5 nmol calcium is taken up per minute per mg protein. Assuming the nitrendipine binding sites equal the number of functioning calcium channels, about 50,000 calcium atoms pass through each channel per minute.

156.12 PHOTOACTIVATED CYCLIC NUCLEOTIDES PROBE THE KINETICS OF CALCIUM CHANNEL REGULATION IN HEART. Joel Nargeot*, Jeanne M. Nerbonne, Henry A. Lester, and Joachim Engels*. Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125.

The positive inotropic and chronotropic effects of beta-adrenergic agonists on heart arise predominantly from an enhanced slow inward current (this current is carried mainly by calcium ions). Binding of agonists to myocardial beta receptors causes an increase in intracellular cAMP which, in turn, is thought to mediate the slow inward current via a chain of events including protein phosphorylation and leading to an increase either in the number of functional calcium channels or of their elementary conductance. The effects of adrenaline are observed after a latency of 4-6 sec and require about 60 sec to reach completion in frog heart at 25°; little is known of the rates of the intermediary biochemical events. Furthermore, the negative inotropic effects of muscarinic agonists are based on a decrease in this calcium current, as well as on an increased potassium conductance. Among the postulated intracellular second messengers for the muscarinic response are decreased cAMP and increased cGMP.

To obtain more information on these events, we are exploiting photoactivatable cAMP and cGMP analogues. The ortho-nitrobenzyl esters of cAMP and cGMP cleave efficiently upon irradiation and therefore allow precise concentration jumps that are complete within at most 300 msec. We are studying the behavior of the slow inward current after such jumps. In voltage-clamped TTX-treated atrial trabeculae from bullfrog heart, flash-induced concentration jumps (1-10 μM) of intracellular cAMP increase the calcium current by at least twofold. The decrease in the slow inward current produced by 1 μM carbachol is completely overcome by cAMP jumps of ~10-20 μM. Furthermore, when this current has been attenuated by 0.1 μM carbachol, a single cAMP jump (~10 μM) results in a five to sixfold increase in the slow inward current. The effects are complete within 30 sec and proceed along a roughly exponential time course, with a time constant of 15-20 sec. Increases are detectable within 300 msec after the flash. Neither the slow inward current nor the muscarinic potassium conductance are affected by concentration jumps of cGMP (up to 100 μM). We conclude that the response to beta agonists is significantly limited in rate either by the activation of protein kinase or, more likely, by the rate of protein phosphorylation.

156.13 BARBITURATES DECREASE VOLTAGE-DEPENDENT CALCIUM CONDUCTANCE OF MOUSE NEURONS IN DISSOCIATED CELL CULTURE

R.L. Macdonald and M.A. Werz. Neurosciences Program and Dept. of Neurology, The University of Michigan, Ann Arbor, MI 48109.

Barbiturates decrease presynaptic release of neurotransmitter, synaptosomal calcium uptake and the duration of somatic calcium-dependent action potentials (CAPs) at anesthetic concentrations. To investigate the mechanism by which barbiturates decrease neuronal calcium entry, we studied barbiturate actions on CAPs and voltage-dependent inward calcium currents evoked in dorsal root ganglion (DRG) and spinal cord neurons grown in primary dissociated cell culture using standard intracellular recording and single electrode voltage clamp techniques. DRG neurons were used in the voltage clamp experiments since they are spherical and do not have the extensive dendritic arborization of spinal cord neurons.

DRG and spinal cord neuron somatic CAPs were decreased in duration by pentobarbital (PB)(100-500 μM) and phenobarbital (PhB)(500-2000μM), while the inactive barbiturate, barbituric acid (BA), was ineffective at 2 mM. CAP duration was decreased in the absence of an effect on resting membrane potential or conductance. PB and PhB also decreased CAP duration of DRG neurons following substantial blockade of potassium conductances by substitution of cesium (Cs) for potassium in the recording medium and by intracellular injections of Cs from recording micropipettes filled with 3 M CsCl or 4 M CsAc. CAPs recorded with Cs injection were much prolonged in duration (>500msec) and retained no after-hyperpolarization, suggesting that most potassium conductance was blocked. DRG neurons were voltage clamped in potassium-free medium containing Cs and using 3M CsCl recording micropipettes. Depolarizing voltage commands (20-80 mV) from a holding potential of -60 mV evoked net inward currents (I_I). Both PB and PhB but not BA decreased the magnitude of I_I. The calcium channel blocker, cadmium (Cd) (500 μM) also blocked I_I. PB did not affect leak current (determined in the presence of cadmium). When potassium conductances were only partially blocked by tetraethylammonium (5mM), both the early I_I and the late outward current were reduced by PB, suggesting that the barbiturates did not enhance calcium-activated potassium conductance.

Thus, it appears that barbiturates decrease calcium entry by decreasing voltage-dependent calcium conductance. It is likely that this mechanism underlies barbiturate presynaptic actions to decrease presynaptic calcium entry and neurotransmitter release.

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156.14 CALCIUM ENTRY MAY INDUCE PROLONGED REFRACTORINESS IN THE BAG CELL NEURONS OF APLYSIA. L.K. Kaczmarek. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Following brief electrical stimulation the bag cell neurons of Aplysia generate a long lasting afterdischarge which, in vivo, serves to trigger egg laying behavior. At the end of their discharge these neurons enter a prolonged refractory period during which further electrical stimulation either fails to elicit afterdischarge or generates only short discharges. This period is also associated with the failure of action potentials which originate in bag cell neurites to invade the somata. Refractoriness endures for about 18-24 hours after which a full length afterdischarge may again be stimulated.

The hypothesis that calcium entry during an afterdischarge triggers the onset of the refractory period was suggested by ion substitution experiments (Kaczmarek et al., Brain Res., in press). In the present study this hypothesis has been further tested using the calcium ionophore Ro2-2985. Incubation of abdominal ganglia for 20 min in an artificial seawater medium in the presence of 5-50 μM Ro2-2985, followed by electrical stimulation of a pleuroabdominal connective, resulted either in a failure to generate afterdischarge or in afterdischarges that were significantly shorter than the control duration (30 min). The mean durations of afterdischarges, recorded with extracellular suction electrodes over the bag cell clusters, were 20.0, 14.0, 1.7, 2.7, and 0.0 min for ionophore concentrations of 1.0, 2.5, 5.0, 10.0, and 50.0 μM, respectively. Failure to generate afterdischarge was usually associated with the failure of action potentials to fully invade bag cell somata. These concentrations of ionophore were not toxic to the cells, since, following exposure to 50 μM Ro2-2985, vigorous afterdischarges could be elicited after extracellular addition of 90 mM TEA, a procedure that is also effective in overcoming the refractoriness that follows an afterdischarge. Recovery from exposure to the ionophore (5 μM) was tested in three ganglia, 20 hrs after washout of the ionophore. In all three cases, electrical stimulation triggered afterdischarges (mean duration 19.0 min). The ionophore (5 μM) was also tested in a calcium-free, TEA containing extracellular medium in which stimulation normally triggers brief (<1 min) bag cell afterdischarges which show no subsequent refractory period. No effects were observed on afterdischarges in this medium.

Brief exposure to Ro2-2985 therefore induces an electrical state in the bag cell neurons that is similar to the refractory state that follows afterdischarge in a calcium containing medium and supports the hypothesis that calcium entry during afterdischarge induces the subsequent refractoriness.

157.1 ADENOSINE INHIBITS EXCITATORY SYNAPTIC TRANSMISSION IN THE IN VITRO RAT HIPPOCAMPUS. W. R. Proctor and T. V. Dunwiddie. Dept. of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262

Adenosine, adenine nucleotides, related purines such as inosine, and metabolically stable analogs of adenosine have been shown to reduce synaptic efficacy in the rat hippocampus in vitro (Dunwiddie and Hoffer, Br. J. Pharm. 69:59, 1980). We have compared the actions of adenosine on extracellularly recorded field EPSPs with its effects on intracellularly recorded potentials in this brain region.

Adenosine (5-100 uM) was found to reduce the amplitude of synaptic potentials elicited by stimulation of excitatory Schaffer collateral and commissural afferents to the CA1 region. Quantitative analysis of the dose-response curve for adenosine on extracellularly recorded EPSPs gave an EC_{50} value of 18 uM (95% confidence limits: 13-25 uM). Intracellular recording from hippocampal pyramidal neurons revealed parallel changes in synaptic potentials during superfusion of slices with adenosine. The decrease in intracellularly recorded EPSPs elicited by adenosine was quantitatively similar to the changes in extracellularly mediated potentials (EC_{50}=21 uM; 95% limits 14-31 uM). When these data were analyzed by means of a Hill plot, the slopes were not significantly different from 1. No significant changes in the resting membrane potential or resistance were found at adenosine concentrations which were sufficient to reduce the EPSP by 50%.

The relatively high potencies of two non-metabolizable analogs of adenosine (L-N^6-phenylisopropyl adenosine, L-PIA; N^6-cyclohexyladenosine, CHA; EC_{50} values less than 0.02 uM) would appear to suggest that these actions are mediated by what have been termed purinergic A_1 receptors. The rank order potencies of purinergic agonists (L-PIA > CHA > NECA > adenosine > ATP > inosine) also supports this classification. Theophylline (as well as other alkylxanthines) was shown to be a competitive inhibitor of the purinergic response when tested at concentrations between 200 uM and 5 mM. K_i values for theophylline estimated from dose-response curves for the various adenosine analogs tested ranged from 39-57 uM; this K_i value correlates well with K_d values previously reported for theophylline (obtained from ligand binding studies on the A_1 receptor).

Thus, a comparison of intracellular and extracellular responses demonstrates that the primary effect of adenosine on hippocampal electrophysiology is an inhibition of excitatory synaptic transmission; little if any change in somatic membrane properties is induced by low concentrations of this nucleoside.

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157.2 ANTAGONISTIC INTERACTIONS BETWEEN ADENOSINE DERIVATIVES AND NOREPINEPHRINE ON BEATING RATE IN RAT ATRIA. M.K. Samet and C.O. Rutledge, Dept. Pharmacol. and Toxicol., School of Pharmacy, Univ. of Kansas, Lawrence, KS 66045.

Adenosine (Ado) and its derivatives can antagonize the rate increasing effect of exogenously administered norepinephrine (NE) in spontaneously beating rat atria (Fed. Proc. 41:1055, 1982). In order to further define this Ado interaction with NE, we have examined a number of Ado derivatives for their efficacy and potency in shifting the concentration-effect curve for NE-induced chronotropy. Since maximal response rate to NE may be a function of basal rate, experiments were performed utilizing carbachol to decrease basal beating rate. Carbachol exposure at 0.2 µM decreased spontaneous beating rate by 50 beats/min but did not change either the EC_{50} or the maximal response rate to NE. Reducing spontaneous beating rate by 170 beats/min with 0.7 µM carbachol produced a slight increase in the maximal response to NE while shifting the EC_{50} by approximately 20-fold. Therefore, all experiments were performed with Ado derivatives at concentrations which produced a maximal 50-60 beats/min depression in spontaneous rate. The A_1-Ado receptor agonist N^6-phenylisopropyladenosine (N^6-PIA) at a concentration of 10 nM was capable of increasing the EC_{50} for NE about 6-fold without altering the maximal change in beating rate. Adenine (100 µM) produced no change in the concentration-effect curve to NE, while inosine (100 µM) decreased the maximal response. Neither compound altered the spontaneous rate. The Ado analog 2'-deoxyadenosine did not change the EC_{50} to NE at either 10 or 100 µM, but produced a decrease in the maximal response rate (similar to 100 µM Ado), while suppressing spontaneous rate by 20 beats/min. The Ado nucleoside derivative S-adenosyl-L-homocysteine (SAH, 100 µM) did not decrease spontaneous rate nor alter the EC_{50} to NE. However, SAH did decrease the maximal response to NE. The apparent A_2-Ado receptor agonist 2',5'-dideoxyadenosine (100 µM) did not alter the EC_{50} to NE, but did decrease the maximal response without altering spontaneous rate. These data demonstrate that A_1-Ado receptors are involved in shifting the postsynaptic sensitivity of atria to NE without affecting the maximal response since only N^6-PIA was capable of shifting the EC_{50} to NE. Moreover, the A_2-Ado receptor agonist 2',5'-dideoxyadenosine decreased the maximal response without changing the EC_{50} for NE. Since adenine did not affect either the EC_{50} to NE nor the maximal response, an intact nucleoside is required for these effects. These data suggest that endogenous Ado may serve a neuromodulatory role in controlling both the potency and efficacy of endogenous NE in increasing atrial rate. (Supported by USPHS Grants NS 12760, NS 16364, 5606 and the Center for Biomedical Research, Univ. of Kansas.)

157.3 SIMILARITIES BETWEEN ADENOSINE RECEPTORS MEDIATING INHIBITION OF NOREPINEPHRINE RELEASE IN KIDNEY AND INHIBITION OF FIELD EPSPs IN HIPPOCAMPUS. B. Fredholm*, T. Dunwiddie and P. Hedqvist*. Depts. of Pharmacology and Physiology, Karolinska Institutet, Stockholm, Sweden and Dept. of Pharmacology, University of Colorado, Denver, CO 80262

Adenosine (ADO) influences sympathetic neurotransmission in the rabbit kidney by causing a prejunctional inhibition and a postjunctional enhancement (Hedqvist & Fredholm, N.S. Arch. Pharmacol. 293:217, 1976). It also inhibits field EPSPs and interictal spikes in the rat hippocampal slice preparation (Dunwiddie, Fredholm and Hoffer, N.S. Arch. Pharmacol. 316:326, 1981). We have further examined the pharmacological specificity of these two effects.

Rabbit kidneys with an intact nerve supply were perfused in vitro with a saline medium containing dextran. The nerve stimulation-induced release of tritium (6 Hz, 90 pulses) from the ^{3}H-NE labelled tissue was determined. ATP, ADP, AMP, and ADO were virtually equipotent in reducing tritium release, β,γ-methylene-ATP was less potent than ADO, and inosine and hypoxanthine were essentially inactive. As an antagonist of the ADO effect, 8-phenyl-theophylline was more potent than theophylline, which was more potent than caffeine (K_i: 0.15, 50, 125 µM respectively). The rank order potency of agonists and the antagonism by methylxanthines suggests the response is mediated by P_1 rather than P_2 purine receptors in terms of Burnstock's classification. The effect of ADO was enhanced in the presence of an adenosine deaminase inhibitor, and by two inhibitors of adenosine uptake.

Additional ADO analogs were used to provide evidence for other purine receptor classification schemes. The adenosine analog L-PIA was about 30 times more potent than ADO (IC_{50}: 0.05 and 2.0 µM respectively). Another adenosine analog NECA had an intermediate potency (0.2 µM) whereas SQ 22356 and 2-deoxy-ADO were almost inactive. This order of potency corresponds to an R- rather than P-type receptor (Londos, C. and Wolff, J. PNAS 77:2551, 1977).

The inhibitory effect of adenosine analogs on field EPSPs in rat hippocampal slices was studied as well. The EC_{50} values for two N^6-substituted analogs, L-PIA and cyclohexyl-ADO, were approximately 0.02 µM, while NECA was about 10-fold less potent. Theophylline was able to competitively antagonize the response to adenosine analogs; in these experiments, the K_i value for theophylline was found to be 39 µM when tested against ADO, and 57 µM versus L-PIA. These results suggest that the presynaptic receptors mediating inhibition of NE release in the kidney, and the receptors (possibly also presynaptic) mediating inhibition of field EPSPs in rat hippocampus are of the A_1 subtype.

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157.4 CHARACTERISTICS OF THE ADENOSINE INHIBITED ADENYLATE CYCLASE (A_1 RECEPTOR) IN RAT AND MOUSE CEREBELLUM. W. J. Wojcik and N. H. Neff. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032.

Adenosine has been found to interact with two different membrane bound receptors. Micromolar concentrations of adenosine and various analogs stimulate the low affinity A_2 receptor and activate adenylate cyclase activity, while nanomolar concentrations inhibit adenylate cyclase through the A_1 receptor. We report that L-phenylisopropyl adenosine (PIA) inhibited basal adenylate cyclase activity in a washed P_2 preparation from cerebellum of rat and mouse. The extent of inhibition was greatest in the cerebellum when compared to the hippocampus, frontal cortex and striatum of the rat. Further studies in the cerebellum indicated the inhibition of adenylate cyclase to be mediated through the A_1 receptor. Nanomolar concentrations of PIA (5 nM to 1 µM) inhibited the basal adenylate cyclase in a concentration dependent manner with the EC_{50} being 60-70 nM. In the presence of 1 µM PIA, a concentration which produces maximal inhibition, basal adenylate cyclase was reduced by 30-40%. We also observed that the adenosine receptor antagonist, isobutyl methylxanthine, 50 µM, competitively antagonized the PIA response by shifting the PIA dose response curve to the right.

In order to associate the A_1 receptor to a specific cell type in the cerebellum, we tested whether selected cerebellar lesions affected the PIA inhibition of adenylate cyclase. Climbing fibers were destroyed by a systemic administration of 3-acetylpyridine (75 mg/kg). Two weeks after this treatment, we tested the responsiveness of adenylate cyclase to PIA (5 nM to 1 µM). We obtained similar dose response curves for PIA in the control and 3-acetylpyridine treated rats. This indicated that the A_1 receptor was not associated with the cerebellar climbing fibers. The neurological mutant, 'Staggerer' mouse, was reported to have no apical dendrites on the Purkinje cells and fewer granule cells. One month after birth, the response of adenylate cyclase to PIA was investigated in the 'Staggerer' mouse cerebellum and in their heterozygous, wild-type control. The basal adenylate cyclase activities were the same for both groups. The EC_{50} for the two dose response curves were also similar. However, the maximal response to 1 µM PIA was different. In the cerebellum of the 'Staggerer' mouse, PIA inhibited basal adenylate cyclase activity by only 19%, while a 41% inhibition was observed in the control cerebellum. This difference in the extent of inhibition may indicate that the A_1 receptor is associated with the Purkinje cell dendrites and/or the granule cells in the cerebellum.

157.5 BARBITURATES INHIBIT ^{3}H-ADENOSINE UPTAKE AND ENHANCE ^{3}H-ADENOSINE RELEASE IN RODENT WHOLE BRAIN SYNAPTOSOMES. R. A. Gonzales* and S. W. Leslie. Dept of Pharmacology, Univ. of Texas Coll. of Pharmacy, Austin, Tx 78712 and Dept. of Pharmacology and Neurosciences Program, Univ. of Alabama in Birmingham, Birmingham, Al 35294

Adenosine has been attracting attention recently for its possible role in mediating inhibition of synaptic transmission in the CNS. We have investigated the effects of barbiturates on adenosine movements across the synaptic plasma membrane using rodent whole brain synaptosomes to test the hypothesis that some of the depressant actions of these drugs may be mediated through interference of an endogenous adenosine system. Adenosine uptake was studied using synaptosomes prepared from male Sprague Dawley rats and Swiss Webster mice by differential and Ficoll gradient centrifugation methods. After preincubation at 37°C, ^{3}H-adenosine was added to the synaptosomes in the presence of pentobarbital, methohexital, and 5-(2-cyclohexylideneethyl)-5-ethylbarbituric acid (CHEB) at varying concentrations and times. The uptake was halted by diluting the synaptosomes in isotonic buffer followed by rapid filtration through Whatman GF/B fiberglass filters. All three compounds significantly inhibited ^{3}H-adenosine uptake at anesthetic concentrations (100 µM-300 µM). Kinetic analysis of the inhibition by pentobarbital showed that it was noncompetitive. In addition, pentobarbital did not affect the distribution of adenosine metabolites in the synaptosomes. Release of adenosine was studied using the P_2 pellet from CD-1 mice. After loading with ^{3}H-adenosine, synaptosomes were filtered, washed, and then incubated with releasing medium which was changed every minute. 50 mM KCl added to the medium caused an enhancement of ^{3}H efflux mainly due to increased release of adenosine and inosine. This effect was abolished in the presence of 250 µM EGTA. 0.3 mM pentobarbital did not significantly alter total release of ^{3}H stimulated by 50 mM KCl, but it caused a 50% increase in ^{3}H-adenosine release. These results suggest some of the depressant effects of barbiturates may be due to a drug induced enhancement of adenosine levels in the synapse.

157.6 ADULT AND NEONATAL MONOAMINE LESIONS PRODUCE DIFFERING LOCOMOTOR RESPONSES TO CAFFEINE. L. Erinoff*, M. Basura*, and S.R. Snodgrass. Neurology Res., Childrens Hospital of Los Angeles, L.A., CA 90054.

Adult rats (270g) were given 20 mg/kg desmethylimipramine (DMI) followed by intraventricular injection of saline-ascorbate, 6-hydroxydopamine (6HDA) 250 ug/20ul, or 5,7-Dihydroxytryptamine (5,7DHT), 150ug/20ul. Three days later, the procedure was repeated except that the opposite ventricle was injected and the 5,7DHT injection was reduced to 75ug/10ul. Two weeks later, one hour recording of locomotor activity was begun using photocell cages. Locomotor activity of control, 6HDA, and 5,7DHT animals were similar, and all rats showed a similar inverted U-shaped dose response function for caffeine (5, 15, and 30 mg/kg). L-phenylisopropyl adenosine (PIA) in doses of 0.05, 0.1, and 0.2 mg/kg reduced the locomotion of all three groups. A dose of 5 mg/kg caffeine reversed the activity decreases associated with 0.1 mg/kg PIA but not 0.2mg/kg.

These data suggest that caffeine increases locomotor activity in adult-treated control, 6HDA, and 5,7 DHT rats by blockade of adenosine receptors. Stimulation of these receptors by low doses of the adenosine agonist l-PIA decreases locomotor activity.

A parallel experiment involved neonatal rats who received DMI followed by intraventricular 6HDA, 100ug/10ul, 5,7DHT, 50ug/10ul, or vehicle on days 3 and 6 postnatal. Rats with neonatal 5,7DHT showed an enduring decrease in locomotor activity. Caffeine (15 mg/kg) increased the activity of DHT rats to equal that of controls. One interpretation of these results is that DHT lesions change the sensitivity of adenosine receptors if made in neonatal rats but not adults.

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157.7 AN ANIMAL MODEL FOR CNS CAFFEINE INTOLERANCE. Mitsutaka Nakamura John M. Carney and H. Dix Christensen, Univ. Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73190.

Caffeine sensitization produced by chronic L-N^6-Phenylisopropyladenosine (PIA) may be an animal model of CNS caffeine intolerance in humans. Six male Sprague Dawley rats were trained to respond under an alternating component schedule in which a 10 min Time Out period preceeded each of four 10 min differential reinforcement of low rates (DRL) 15 sec components. Cumulative dose-effect curves for caffeine (3.2-56 mg/kg) provided stimulation of responding and a reduction in reinforcement at relatively low doses in 4 of the 6 rats. No dose of caffeine produced stimulation in the other 2 rats. Caffeine caused a generalized decrease in DRL behavior at higher doses (32 and 56 mg/kg). L-PIA (0.01-0.178 mg/kg) only produced dose-related decreases in responding. Daily injection of 1.0 mg/kg L-PIA resulted in the development of caffeine sensitization to the behavioral stimulant effects of caffeine, with all six rats now showing stimulation. Chronic L-PIA injection resulted in a 10 fold increase in the maximum amount of DRL response stimulation. Other caffeine behavioral responses are not altered. In the process of developing PIA tolerance one would anticipate the alteration to be associated with the high affinity adenosine A_1 receptor, but there could be some disposition differences. In CNS caffeine intolerant humans, plasma kinetics, overt metabolism and cardiovascular responsiveness are within the ranges of non-smoking caffeine abstainers.

Cortical cups were implanted in these rats to investigate drug disposition by both superfusion and affusion. Simultaneous measurement of behavior and caffeine or PIA extraneuronal concentrations can be determined. In drug free animals both PIA and caffeine superfusate concentrations were about 5% of that in plasma. In the case of caffeine, affusate concentrations could approach those of cortex tissue homogenates. PIA, measured in the affusate, gave anticipated tissue concentration for behavioral response that are only slightly higher than that required in binding studies. The results from these studies gives a working hypothesis that CNS caffeine intolerance may be due to altered adenosine receptors. The PIA tolerant rat provides a model in which to study changes in adenosine receptor systems.

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157.8 DISCRIMINATIVE PROPERTIES OF METHYLXANTHINES. F.A. Holloway and H.E. Modrow* Dept. Psychiatry and Behavioral Sciences, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190 U.S.A.

Recently we reported that rats trained to discriminate caffeine from saline in an appetitive operant task displayed generalization of the caffeine training cue to another xanthine, theophylline, but not to several other stimulants, including amphetamine, methylphenidate, or nicotine. We now assessed cross generalization among xanthines and examined blockade of the xanthine cue with 1-phenylisopropyl-adenosine (PIA) or a benzodiazepine, chlordiazepoxide. The latter drugs are of interest since caffeine has been shown to impair CNS binding of both PIA and diazepam.

In a two-lever operant task, male albino rats were trained to press one of the two levers for food pellets after saline injections and the other after injections of either 32 mg/kg caffeine or 56 mg/kg theophylline. Reinforcement was available on a variable ratio schedule (VR 10) for correct responses. Training sessions lasted 10 minutes and were preceded by a one-minute extinction period. After attainment of criterion performance ($\geq$ 70% accurancy on 8 consecutive days), generalization tests were given every 3-4 days. Each test consisted of a single 2 minute extinction period. Drugs were injected 20 minutes prior to test or training sessions. Both response rate and discrimination accuracy (% drug-lever response) were recorded.

Caffeine and theophylline trained rats showed generalization to the training drug and cross-generalization to each other. Strong generalization of the caffeine cue to paraxanthine (one of the major metabolites of caffeine in rats) was evident. Discrimination accurancy increased and reponse rate decreased with increases in dose. Little generalization of either xanthine cue to amphetamine was noted. Both the caffeine and theophylline cues were largely blocked by 5 or 10 mg/kg chlordiazepoxide but neither cue was significantly affected by 0.1 mg/kg PIA, a dose which markedly depressed response rate. These data suggest that the cue properties of xanthines may be based on their action at the putative benzodiazepine receptor. Nevertheless, the relationship among xanthines, benzodiazepines, and adenosine is clearly a complex one. For example, prior work has shown that the adenosine cue itself can be blocked by caffeine. We found no information on caffeine's possible antagonism of the benzodiadepine cue.

158.1 BRAIN MEMBRANE AFFINITY COLUMN: AN APPROACH FOR ENDOGENOUS BENZODIAZEPINE RECEPTOR LIGAND PURIFICATION. D. Urquhart and A. K. Sinha. Dept Physiology & Biophysics, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854

Our focus has been to characterize a ^{3}H-diazepam displacing activity present in the 105,000 x g supernatant of hamster brain (Br.Res.193:519(1980)). We have determined this material to be nondialyzable and < 20,000 daltons as indicated by ultrafiltration (Amicon). The stability of this substance to heating at 95° C for 60 min. is dependent upon the homogenization medium. It is thermostable in .32 M sucrose and thermolabile in 50 mM Tris HCl (pH = 7.4). Ammonium sulfate precipitates this material within 20-56% of saturation.

In an effort to purify this ^{3}H-diazepam binding inhibitor we have prepared an affinity column composed of 30,000 x g brain membrane. Celite forms the supportive matrix of this column. ^{3}H-diazepam binds to this column and can be displaced by an excess of cold. When a solution of 20 μM diazepam is applied to a column preloaded with 2 nM ^{3}H-diazepam, ∿ 50% of the radioactivity is eluted within 1.5 column volumes. This elution pattern contrasts to that obtained when buffer, (Tris HCl, pH 7.4), alone is the eluant. Buffer washes out ^{3}H-diazepam without a sharp peak of radioactivity. There is no indication that Celite itself specifically binds diazepam. As yet, the pharmacological specificity of such "diazepam displaceable" binding needs to be determined.

Brain membrane contains many different binding "sites". Therefore, to harvest a specific endogenous ligand one must choose a specific eluant. As a result, the eluting substance must be separated from the endogenous ligand. We plan to exploit the nondialyzable nature of this endogenous ligand and use its affinity for the receptor to purify it.

158.2 INHIBITION OF BENZODIAZEPINE RECEPTOR BINDING IN RAT BRAIN BY β-CARBOLINE 3-CARBOXAMIDES. R.A. Locock*, G.B. Baker, R.G. Micetich and R.T. Coutts*. Neurochem. Res. Unit, Univ. of Alberta, Edmonton, Canada, T6G 2G3.

A series of tetrahydro β-carboline 3-carboxamides have been synthesized by condensation of D- and L-tryptophan with formaldehyde, methyl ester formation and condensation with aliphatic amines. The corresponding aromatic β-carboline derivatives were synthesized by aromatization of methyl tetrahydro β-carboline 3-carboxylate and condensation with the appropriate amines. These amides are related structurally to ethyl β-carboline 3-carboxylate (β-CCE) which has a high affinity for benzodiazepine receptors (Braestrup et al., Proc. Natl. Acad. Sci., 77, 2288-2292, 1980).

The inhibition of specific ^{3}H-flunitrazepam binding to rat cortical P_2 synaptosomal suspensions prepared according to Klepner et al. (Pharmacol. Biochem. and Behav., 11, 457-462, 1979) was measured by a filtration assay. The P_2 synaptosomal membranes (0.5 mg protein/ml) in 0.1M tris citrate (pH 7.1) were incubated at 0°C for 1 hour with 1 nM ^{3}H-flunitrazepam and the compounds under test. Binding was terminated by rapid filtration under vacuum through Whatman GF/B filters. After washing twice with ice-cold buffer the radioactivity remaining on the filter was estimated by conventional liquid scintillation counting. Non-specific binding was estimated in the presence of 1 μM clonazepam.

IC_{50} values were calculated by log-probit analyses of five concentrations of the inhibitors in duplicate. The most potent compound was ethyl β-carboline 3-carboxamide with an IC_{50} value of 245.9 ± 10.5 nM/liter (n=5). Increasing the number of carbon atoms in the side chain decreased potency, e.g. n-butyl β-carboline 3-carboxamide gave a value of 715.3 nM/liter. In the tetrahydro β-carboline series the most potent compound was dimethyl tetrahydro β-carboline 3-carboxamide, IC_{50} = 64.8 ± 9.7 μM/liter. Again, increasing side chain length was accompanied by decreasing potency.

Although these amide derivatives are less potent inhibitors of benzodiazepine binding than β-CCE (IC_{50} = 3.5 nM, by this procedure), the β-carboline 3-carboxamides may be more effective in vivo since they may not be as rapidly metabolized as the β-carboline 3-carboxylate esters.

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158.3 ANXIOGENIC POTENTIAL OF β-CARBOLINE AND RELATED COMPOUNDS AS BIOASSAYED BY GENERALIZATION TO INTEROCEPTIVE DISCRIMINABLE STIMULI (IDS) PRODUCED BY PENTYLENETETRAZOL (PTZ). H. Lal, D. Bennett*, F. Elmesallamy* and T. Gherezghiher. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

The occurance of β-carboline in mammalian tissues and their ability to bind to the benzodiazepine receptors suggest that these compounds could be endogenous ligands for the benzodiazepine receptors. However, β-carbolines lack benzodiazepine-like pharmacological profile in vivo. It is therefore, possible that β-carbolines produce effects opposite to those of benzodiazepines in humans, i.e. induction of anxiety. To explore this possibility, we tested these compounds for generalization to the IDS produced by PTZ. These IDS are generalized only to drugs that are anxiogenic in man and are blocked by specific anxiolytic drugs (Lal and Shearman, Ann. Rev. Med. Chem. 15: 51, 1980). We trained food-deprived, male-hooded rats to discriminate PTZ-induced IDS from saline by selecting one lever after injection of PTZ and another lever after injection of saline on a fixed-ratio-of-ten schedule of food reinforcement. After the discrimination of PTZ-cue was reliably established, β-carbolines were tested. These rats selected PTZ-appropriate lever after injection of β-carboline, harmane or harmaline but not after injections of diazepam. PTZ, harmane and harmaline were equipotent. PTZ and harmane showed similar efficacy. Doses higher than those producing 50% efficacy could not be tested in cases of β-carboline and harmaline as those doses suppressed responding. Diazepam given along with the test drugs antagonized the IDS produced by PTZ and harmane. Diazepam combination with β-carboline and harmaline resulted in behavioral toxicity such that the discriminative responses were not emitted. Since drug discrimination is a form of animal behavior which closely parallels verbal reports of drug action in human, it is suggested that β-carbolines and related chemicals are anxiogenic pharmacological agents.

158.4 DIFFERENTIAL MODIFICATION OF GABA AND BENZODIAZEPINE BINDING SITES BY GROUP SELECTIVE REAGENTS. M.K. Ticku[1,2] and T.P. Burch[1]*. Dept. Pharmacology[1] and Psychiatry[2], Univ. Tex. Hlth. Sci. Ctr., San Antonio, TX 78284

The effect of group-selective reagents on the binding of [^{3}H]-benzodiazepines and its enhancement by muscimol was investigated in rat brain membranes. Diethyl pyrocarbonate (DEP) and diazotized sulfanilic acid produced a dose-related (0.1-10 mM) inactivation of [^{3}H]diazepam (1 nM), [^{3}H]flunitrazepam (0.3 nM) and [^{3}H]propyl-β-carboline-3-carboxylate (0.3 nM) to cortex, cerebellum and hippocampus. DEP and sulfanilic acid produced inactivation of 50% of the binding sites for the three ligands at 1-1.5 mM and 0.5-1.0 mM, respectively. Scatchard analysis revealed the DEP (1 mM) produced a decrease in the B_{max} (~40-60%) of these three radioligands in the three regions tested without altering the K_D. Partial inactivation of [^{3}H]diazepam binding by 1 mM DEP in cortex, cerebellum or hippocampus did not alter the ability of muscimol and pentobarbital to enhance [^{3}H]diazepam binding in any of these regions. The EC50 values for muscimol or pentobarbital were similar in control and DEP (1 mM) treated regions. In contrast, following the inactivation of [^{3}H]diazepam binding with sulfanilic acid, muscimol or pentobarbital were not able to enhance [^{3}H]diazepam binding. The effect of these two group-selective reagents on [^{3}H]GABA binding indicated that while DEP did not alter [^{3}H]GABA binding significantly, sulfanilic acid was a potent inhibitor of [^{3}H]GABA binding. Thus, while both DEP and sulfanilic acid inactivates the benzodiazepine binding sites, sulfanilic acid also eliminates [^{3}H]GABA binding and the enhancement of [^{3}H]diazepam binding by GABA agonists. These results further support the notion that benzodiazepines and GABA bind to two distinct sites.

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158.5 PHOSPHORYLATION OF GABA-MODULIN IN RAT BRAIN CORTICAL SLICES, SYNAPTOSOMES, AND SYNAPTIC MEMBRANES. B.C. Wise*, A. Guidotti and E. Costa. (SPON: W. Mendelson). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

GABA-modulin (GM), a brain membrane bound protein of MW 16,000 that inhibits the binding of ^{3}H-GABA to its recognition site and inhibits the stimulation of ^{3}H-diazepam binding by GABA, has recently been purified to homogeneity and shown to be a substrate for cAMP-dependent protein kinase. In the present studies, GM was found to be present in a phosphorylated state in rat cortical synaptosomes and slices which had been incubated with inorganic ^{32}P. After the incubation, GM was extracted from the tissue preparations with 1 N acetic acid (80°C) followed by ammonium sulfate precipitation and reverse-phase HPLC. Liquid scintillation spectrometry of the HPLC fractions revealed a peak of radioactivity that corresponded to the GM protein peak. GM was also phosphorylated in crude cortical synaptic plasma membranes that were incubated with Ca^{2+}, calmodulin, phosphatidylserine, or cAMP. GM was extracted and phosphorylation quantitated using the same procedure as that for the intact tissue. Ca^{2+} caused a 2-fold increase in GM phosphorylation, while calmodulin (0.65 µM) and phosphatidylserine (25 µg/ml) in the presence of Ca^{2+} caused a further increase in GM phosphorylation by 5- and 2-fold, respectively. cAMP (50 µM) was less effective (1.5-fold increase) and cGMP (50 µM) was completely ineffective in supporting GM phosphorylation under the experimental conditions used (2 min incubation at 30°C). The incorporation of ^{32}P into the GM molecule was confirmed by SDS-polyacrylamide gel electrophoresis and liquid scintillation counting of slices cut from the gel. We are investigating whether the phosphorylation of this membrane bound protein is important in the regulation of the GABA-benzodiazepine receptor complex.

158.6 ACTION OF BETA-CARBOLINE IN FLUNITRAZEPAM-PHOTOLINKED CULTURES C.Y. Chan*, T.T. Gibbs*, and D.H. Farb* (SPON: D. Soifer). Dept. of Anatomy & Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY 11203.

Benzodiazepines (BZDs) enhance GABA chemosensitivity and potentiate GABA-like synaptic activity in cell cultures of embryonic chick spinal cord. After UV-irradiation in the presence of 100 nM flunitrazepam (FNZM), both BZD binding and enhancement of GABA chemosensitivity were specifically reduced by 75%. The GABA response in the absence of BZD was unaltered, indicating that photoinactivation of the BZD site does not also inactivate the GABA receptor. About 25% of BZD binding sites were resistant to inactivation and presumably account for the residual enhancement of GABA action in inactivated cultures. All neurons studied exhibited some residual BZD sensitivity, indicating that inactivation-resistant sites are not limited to a neuronal subpopulation. There was no apparent change in BZD binding affinity or biphasic dissociation kinetics after inactivation of membranes, indicating that inactivation-sensitive and -resistant sites are otherwise similar. Recent studies have shown that β-carbolines compete with BZDs for CNS binding sites and elicit anxiety-related behavior effects. We have studied the effects of β-carboline carboxylate methyl ester (BCCM) upon the electrophysiological response to GABA in cell culture and the binding of ^{3}H-FNZM to CNS membranes before and after photoaffinity inactivation of benzodiazepine sites. Standard intracellular microelectrode techniques were used to measure membrane potential and conductance of embryonic chick spinal cord neurons in culture. Multiple pressure ejection pipets were used to apply drugs to individual neurons. BCCM had no effect when applied alone (5nM-1µM), but when applied prior to GABA, BCCM inhibited GABA-induced conductance changes in a dose-dependent manner, with maximal inhibition of about 45% and an EC_{50} of 5-10 nM. This is in good agreement with the IC_{50} of 10nM which we have found for competition of BCCM with ^{3}H-FNZM in membrane homogenates. In the presence of 1µM BCCM, the response to 17µM GABA was reduced by 47%, while the response to 100µM (saturating) GABA was reduced by 43%. Thus, BCCM appears to act as a partial noncompetitive antagonist of GABA action. Although BZD binding and enhancement of GABA chemosensitivity were reduced by 75% in photoinactivated cultures, inhibition of the GABA response by 1µM BCCM was unaltered (51% vs. 53% in control cultures). Thus, photoinactivation does not affect the inhibitory action of BCCM, indicating that the site of BCCM action is either distinct from the BZD site or included in the inactivation resistant fraction of BZD sites. (Supported by NSF BNS-80-04871, NIH NS 18536, and MDA fellowships to T.T.G. and C.Y.C.).

158.7 BENZODIAZEPINE RECEPTOR TURNOVER IN CELL CULTURE. L. A. Borden*, C. Y. Chan* and D. H. Farb* (SPON: D. A. Fischman). Dept. of Anatomy & Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY 11203.

Saturable, high-affinity binding sites for benzodiazepines (BZDs) have been identified in homogenates of CNS tissue and in cell cultures prepared from brain or spinal cord. The level of brain BZD binding sites is regulated by convulsive activity like that observed in epilepsy and possibly by chronic exposure to BZD drugs. While neural activity appears to play a role in BZD-R regulation, it is totally unknown as to what control mechanisms and/or chemical signals might underlie such effects. As a first step, we have developed methods, both biochemical and electrophysiological, to monitor BZD-R turnover. When embryonic chick brain or spinal cord cell cultures were photolinked with flunitrazepam (FNZM), washed and scraped, the reversible binding of (^{3}H)FNZM to P_2 membranes was decreased to ca. 75% of control, non-irradiated cultures. Photolabeling of spinal cord cultures resulted in a 75% decrease in the maximum BZD potentiation of GABA chemosensitivity (α_{max}=% potentiation) demonstrating that photoaffinity binding labels the functional BZD-receptor (BZD-R). To study recovery in culture, receptors were photoblocked with unlabeled FNZM (100nM, 0°C, UV for 20 min); control dishes were kept in the dark. Cultures were washed and then scraped and homogenized either immediately, or after various times of incubation. Reversible binding of 5nM (^{3}H)FNZM, determined by filtration, increased with recovery time ($t_{1/2}$~8.5 hr). The electrophysiological response (α_{max}=441%, n=8) returned to control values (α_{max}=444%, n=14) by 24 hr after photoaffinity blockade (α_{max}=123%, n=11) showing that the recovery of functional BZD-R is being measured. Cycloheximide (20µg/ml) prevented the return of binding sites, suggesting that recovery is dependent on protein synthesis. To study receptor degradation, cultures were photolabeled with 5nM (^{3}H)FNZM for 20 min at 0°C (+ or - 1mM flurazepam). Cultures were washed and returned to the incubator for various periods of time, then scraped, homogenized and radioactivity in the pellet (30,000 x g, 20 min) was determined. Irreversibly bound radioactivity decreased with a $t_{1/2}$~6 hr. The radioactivity released into the growth media did not comigrate with authentic (^{3}H)FNZM in TLC (chloroform:acetone, 9:1), indicating that loss of bound radioactivity does not represent dissociation, a conclusion also supported by the blockade of recovery by cycloheximide. Further, greater than 90% of the specifically bound radioactivity remained associated with (^{3}H)FNZM photolabeled membranes after dialysis in 1% Triton (24 hr, 4°C). (Supported by NSF BNS-80-04871, NIH NS-18536, and a MDA fellowship to C.Y.C.)

158.8 PHARMACOLOGICAL AND PHYSICAL PROPERTIES OF TWO CENTRAL BENZODIAZEPINE (BZ) RECEPTORS. M. M. S. Lo and S. H. Snyder. Johns Hopkins Uni. Sch. of Med., Dept. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

Two pharmacologically distinct population of central BZ receptors are separated by their solubility to non-denaturing detergents. Extraction of native membranes with various detergent solubilizes only a distinct fraction of the BZ receptors (Lo, M.M.S. et al., Proc. Natl. Acad. Sci. USA, 79:680-684, 1982). The BZ receptors associated with the insoluble pellet is soluble in salt and detergent. The salt plus detergent soluble (SE) BZ receptors display pharmacological properties similar to Type I BZ receptors whereas the detergent soluble (DE) BZ receptors resemble Type II receptors.

Both physical forms are modulated by γ-aminobutyric acid (GABA). Anions stimulate BZ binding in the DE receptors whereas the SE receptors are not regulated by anions. Some divalent cations also regulate the DE BZ receptors but not the SE receptors.

Dissociation kinetics of the two soluble forms of BZ receptors are also different. Monophasic dissociation of [^{3}H]diazepam is observed with DE receptors at 0°C in the presence of 1,000 fold excess diazepam ($K_{-1} = 3.9 \times 10^{-3} s^{-1}$) which is decreased in the presence of chloride. On the other hand, dissociation of [^{3}H]diazepam is clearly biphasic in SE receptors.

Heat inactivation of the two soluble BZ receptors appear to be similar and consists of at least two exponential components. GABA protects both forms of BZ receptors (DE and SE) by reducing the rate of inactivation of the fast and slow phases. However, chloride ions only protects the fast phase of DE receptors. In contrast, calcium ions protects only the slow phase in DE receptors. Both anions and cations does not protect SE receptors from heat inactivation.

We have also characterized the physical properties of the two soluble BZ receptors. Chromatography in HPLC protein sieve columns shows that native DE receptors, labelled with [^{3}H]flunitrazepam, consists of two oligomeric forms with molecular weights about 250,000 and 60,000. In contrast, SE receptors exists only in the low molecular weight form. Separation by reverse phase HPLC shows that the soluble BZ receptors (DE and SE) consists of 3 major peaks which occurs with different stoichiometry in the two types of soluble receptors. This shows that the DE and SE BZ receptors exist in different oligomeric states and contain physically heterogeneous populations which are also different in their pharmacological and kinetic properties.

158.9 CENTRAL BENZODIAZEPINE (BZ) RECEPTORS: HETEROGENEITY AND COOPERATIVITY IN RECEPTOR SUB-POPULATIONS. R.R. Trifiletti*, M.M.S. Lo and S.H. Snyder. (SPON: V.B. Mountcastle). Johns Hopkins University School of Medicine, Depts. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

Kinetic properties of central BZ receptors from bovine brain have been studied in vitro in both the membrane and soluble state. The data are most consistent with the existence of at least two distinct receptor populations and cooperativity in some of the receptor populations.

Dissociation kinetics of [^{3}H]-flunitrazepam (FNZ) or [^{3}H]diazepam (DZ) is biphasic in membrane preparations whether initiated by drug displacement or infinite dilution (for cerebral cortex, k_{-1} = 1.4 x 10^{-3} sec^{-1} and 0.7 x 10^{-3} sec^{-1}, for fast and slow phases respectively). Considerable regional variation is apparent. Pre-incubation with methyl β-carboline 3-carboxylate (Ro-22-7497), selectively abolishes the fast phase of [^{3}H]-DZ or [^{3}H]-FNZ dissociation in a dose-dependent fashion. The ED_{50} (∿1 nM) for this effect is comparable to the K_d of Ro-22-7497 at Type I benzodiazepine receptors.

Analysis for cooperative effects, by comparing dissociation by infinite dilution into buffer or buffer containing various ligands, indicates that the fast phase of [^{3}H]-FNZ dissociation is sensitive to receptor occupation. Agonists such as FNZ or CL 218,872 (all 1 μM) accelerate the fast phase (k_{-1} = 1.9 x 10^{-3} sec^{-1}) while antagonists such as Ro-15-1788 or Ro-22-7497 (all 1 μM) slow this phase (k_{-1} = 0.9 x 10^{-3} sec^{-1}). By contrast, the slow phase of [^{3}H]-FNZ dissociation is unaffected by receptor occupation. Similar experiments with [^{3}H]-ethylβ-carboline 3-carboxylate (CCE), shows a biphasic dissociation curve at 1.0 nM by infinite dilution and prominent cooperative effects. These results suggests that at least some sub-populations of BZ receptors display cooperative interactions with both BZ and β-carbolines.

Differential solubilization [Lo et al., Proc. Natl. Acad. Sci. USA 79:680-4 (1982)] indicates that salt plus detergent soluble (SE) receptors displays biphasic dissociation kinetics of [^{3}H]-FNZ whereas dissociation from detergent soluble (DE) receptors is monophasic. Cooperative phenomena appear to be present only in the SE receptors. This may mean that cooperative phenomena are restricted to Type I receptors, known to be enriched in the SE fraction.

In summary, the kinetic data obtained suggests that the complex kinetic properties of central benzodiazepine receptors observed in the membrane state may be explicable in terms of distinct sub-populations of receptors of different kinetic properties. Cooperative interactions appear present in some of these sub-populations.

158.10 EFFECT OF GABA ON [^{3}H]-PROPYL BETA-CARBOLINE-3-CARBOXYLATE LABELLED BENZODIAZEPINE RECEPTOR SUBTYPES IN RAT BRAIN. K.W.Gee*, F.J.Ehlert* and H.I.Yamamura. (SPON: J.B.Angevine). Dept of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

The regulation of benzodiazepine(BZD) receptor subtypes(type I and type II) by GABA was studied by assessing the effect of GABA on flunitrazepam(FLU) inhibition of [^{3}H]-FLU and [^{3}H]-propyl beta-carboline-3-carboxylate([^{3}H]-PCC) binding in various brain regions. In the hippocampus, the type I BZD receptor(high affinity) labelled by a low concentration(0.04nM) of [^{3}H]-PCC at 0°C did not appear to be GABA regulated. This was based upon the observation that GABA(10^{-4}M) has no significant effect on FLU/[^{3}H]-PCC competition curves in the hippocampus. In contrast, when [^{3}H]-FLU(0.05nM) or a high concentration of [^{3}H]-PCC(0.5nM) was used to label both type I and type II(low affinity) receptors under similar conditions, GABA(10^{-4}M) caused a significant increase(1.7 fold) in the affinity of FLU as measured by FLU/[^{3}H]-FLU and FLU/[^{3}H]-PCC competition experiments. In cerebral cortex, GABA enhancement of FLU inhibition of [^{3}H]-PCC(0.04nM) binding was present but significantly less than the enhancement observed with [^{3}H]-FLU(0.05nM) or a high concentration of [^{3}H]-PCC(0.5nM). An effect of GABA on the potency of FLU was also observed in the cerebellum when either [^{3}H]-FLU or [^{3}H]-PCC was used as ligands. Dissociation kinetics of high(0.5nM) or low (0.04nM) concentrations of [^{3}H]-PCC in the hippocampus support the notion that 0.04nM [^{3}H]-PCC labels a single binding site whereas the high concentration of [^{3}H]-PCC labels both the high (type I) and low affinity(type II) sites. In the cerebral cortex, low concentrations of [^{3}H]-PCC predominantly labels the high affinity site with a small proportion of the low affinity site also being labelled. PCC/[^{3}H]-FLU competition curves in the cerebellum indicate PCC binds to a single BZD receptor site in this brain region. Overall, these findings suggest that GABA regulation may be predominantly associated with the type II BZD site found in the cerebral cortex and hippocampus. In addition, a regional difference in the way type I receptors are associated with GABA receptors may occur. Consequently, GABA regulation of BZD receptor affinity may be a determinant of BZD receptor heterogeneity. (Supported by USPHS grants MH-27257, MH-30626 & Program Project grant HL-20984. NRSA(NS-06923) from the NINCDS to K.W.Gee and RSDA, Type II(MH-00095) from the NIMH to H.I. Yamamura.)

158.11 INFLUENCE OF AGE ON β-CARBOLINE BINDING IN HIPPOCAMPAL SUBFIELDS R.H. Zobrist*, H.L. Komiskey and T.F. Murray. College of Pharmacy, Washington State University, Pullman, WA 99164-6510.

Studies indicate that aging in humans leads to increased sensitivity to the CNS depressant effects of the benzodiazepines(BZ). This increased sensitivity may be related to an increase in BZ binding in the hippocampus (Memo, et al., J. Pharm. Pharmacol. 33: 64, 1981). However, different areas of the hippocampus are known to vary in the type of neurons, neurotransmitter levels and functions (Jones and Smith, Prog. Neurobiol.,15:19, 1980). Therefore, subregions of the hippocampus were examined for an age-dependent alteration in 3H-propyl-β-carboline-3-carboxylate (3H-PCC) binding. 3H-PCC is believed to selectively label a BZ_1 receptor subclass (Braestrup and Nielsen, J. Neurochem., 37:333, 1981). Furthermore, a derivative of the β-carbolines may be related to a presumed endogenous ligand (Braestrup et al., Proc. Nat. Acad. Sci., 77:2288, 1980).

Male Sprague-Dawley rats were decapitated and the hippocampi removed. Tissue from 4 animals was pooled, homogenized in 50mM Tris-Cl buffer (pH 7.3) and centrifuged at 48,000 X g for 10 min. The pellet was resuspended and washed 3 times with ice-cold buffer. Aliquots of the final 1:25 w/v homogenate were then incubated in triplicat with 3H-PCC for 90 min. at 0-4°C. Incubation was terminated by vacuum filtration through Whatman GF/B filters and immediately washed with 3 X 3ml of ice-cold buffer. Binding in the presence of luM unlabeled ethyl-β-carboline-3-carboxylate was defined as nonspecific. Scatchard analysis of equilibrium binding experiments in hippocampal membranes over a 3H-PCC concentration range of 1pM to 12nM indicated a Kd=0.93nM and Bmax=798 fmol/mg protein. In addition, a second binding site of somewhat higher affinity was present.

The subregions of the hippocampus were examined for an age-dependent change in 3H-PCC binding. Young (3-4 months), mature (12-13 months), and old (28 plus months) male Fischer-344 rats were used. After decapitation the hippocampi were removed, subfields (regions CA1 and CA4/area dentata) were dissected out under a 20X dissecting microscope and stored at -80°C until used (within 24 hours of removal). Tissue from 3 animals was pooled and prepared for the binding assay as indicated above. A saturating concentration of 3H-PCC (6nM) was used to determine if the maximum number of 3H-PCC binding sites varied with age. A 12-20 percent reduction in specific 3H-PCC recognition sites was found in the subfields of the hippocampus of the old rats as compared to the corresponding areas of mature and young animals. These results indicate that aging may be associated with a reduction of BZ_1 receptors in specific regions of the hippocampus. (Supported by USPHS Grant AG 03327-01)

158.12 FOMINOBEN: A COMPOUND WITH PROMINENT BENZODIAZEPINE-LIKE ACTIVITY. S.A.Springfield*, B.Krespan*, F.Baldino, P.Skolnick*+ and H.M. Geller (SPON: W.J.Nicklas). Dept. of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854, +NIMH, Washington, DC 20205.

Fominoben (3'-chloro-2'-(N-methyl-N-[(morpholino-carbonyl)-methyl]-aminomethyl)benzanilide-hydrochloride) is a novel therapeutic agent with both antitussive and respiratory stimulant properties. Recent communications have also reported that fominoben can displace ^{3}H diazepam binding from brain membranes. We have now conducted biochemical, behavioral, and electrophysiological experiments to characterize further the actions of this interesting agent. Initially, we examined the ability of fominoben to displace ^{3}H-diazepam from putative benzodiazepine receptors on washed rat cerebral cortical membranes, and replicated the earlier work showing an IC_{50} of 1 μM. We also examined the interaction of fominoben with BCCE in the presence and absence of 10 μM GABA. The IC_{50} of fominoben for displacement of BCCE in the absence of GABA was approximately 6 μM, whereas in the presence of 10 μM GABA the IC_{50} was reduced 38%. The findings would suggest that fominoben has benzodiazepine-like qualities.

Two series of experiments were designed to test this possibility in animals. Fominoben was first tested for its ability to antagonize seizures produced in CF1 mice by either pentylenetetrazol (PTZ, 50 mg/kg) or 3- mercaptoproprionic acid (3-MP, 75 mg/kg). Mice were given i.p. injections of 0.5-100 mg/kg fominoben 30 min prior to i.p. injection with either of the convulsants. We measured time to seizure following PTZ injection. Treatment with 50 mg/kg or 100 mg/kg of fominoben prolonged time to seizure beyond 15 min (the maximum measured). When the selective BDZ antagonist, RO 15-1788, was injected i.p. (5 or 10 mg/kg) 15 min prior to the injection of PTZ, the action of fominoben was blocked. Second, electrophysiological experiments were performed on rat cerebral cortical neurons in situ. Fominoben (1 mg/kg, i.v.) produced a rapid depression of neuronal activity in 2 of 5 cells tested. Additional administration of fominoben (1-2 mg/kg) resulted in a total inhibition of discharge in these 2 cells. In 2 cells fominoben (1 mg/kg) produced an increase in the rate of activity, whereas increasing the dosage above 1 mg/kg resulted in a dose-related decrease. The ability of RO 15-1788 to reverse the depressant action of fominoben was tested on these 2 cells. RO 15-1788 (10 mg/kg i.v.) reversed the depressant action of fominoben on both cells. On one, fominoben (8-16 mg/kg) was again injected after RO 15-1788 and the unit was once again depressed. In summary, the data from biochemical, behavioral and electrophysiological tests are consistent with fominoben being a benzodiazepine-like compound. (Supported by NIH grant NS 15468.)

158.13 COUPLING OF PUTATIVE CALCIUM CHANNELS WITH BRAIN BENZODIAZEPINE RECEPTORS. J.D. Hirsch and R.L. Kochman. Dept. Biol. Res., G.D. Searle & Co., Chicago, IL 60680.

Nickel ion has opposite effects on the specific binding of ^{3}H-diazepam (^{3}H-DZ) and ^{3}H-propylβ-carboline carboxylate (^{3}H-PrCC) to mouse brain benzodiazepine receptors, stimulating the former and inhibiting the latter. With ^{3}H-DZ, Ni^{2+} increased binding by increasing ligand affinity while versus ^{3}H-PrCC, binding affinity declined and the number of sites increased. Since these Ni^{2+} effects are not due to direct chloride channel or GABA receptor interactions and since Ni^{2+} is a calcium antagonist in other systems, we examined the possibility that the effects of this ion were through a Ca^{2+}-associated mechanism. 4-Aminopyridine, which increases Ca^{2+} transport, inhibited ^{3}H-DZ and ^{3}H-PrCC binding with IC_{50}'s of 1650 and 2000μM respectively. This drug competitively inhibited ^{3}H-DZ binding but was a noncompetitive inhibitor of ^{3}H-PrCC binding. 4-Aminopyridine did not displace ^{3}H-DZ from kidney membranes nor did it displace ^{3}H-muscimol from GABA receptors. Displacement of ^{3}H-DZ by 4-aminopyridine was blocked in a concentration-dependent fashion by Ca^{2+}, La^{3+} at 5mM, and Ni^{2+} at 5mM. In this case, Ca^{2+} and La^{3+} had little effect on basal ^{3}H-DZ binding, while Ni^{2+} stimulated binding. Ni^{2+} had no effect on ^{3}H-DZ binding in kidney membranes or on ^{3}H-muscimol binding in brain membranes. The Ni^{2+} stimulation of ^{3}H-DZ binding in brain (55% at 5mM) was decreased in a concentration-dependent fashion by Ca^{2+}; at 5 mM Ni^{2+} and 200 mM Ca^{2+} binding was the same as control values. However, Ca^{2+} could not reverse the Ni^{2+} inhibition of ^{3}H-PrCC binding. Like Ni^{2+}, the other Ca^{2+} antagonist cations, Mn^{2+}, Co^{2+}, and Cd^{2+} all stimulated ^{3}H-DZ binding and inhibited ^{3}H-PrCC binding. Basal binding of ^{3}H-DZ was stimulated 33% by 100μM GABA and 44% by 500μM pentobarbital. These indirect alterations in the benzodiazepine receptor also reduced the affinity of 4-aminopyridine by 20 to 40%. Photoaffinity inactivation of specific ^{3}H-DZ binding in brain membranes, which spared all of the specific ^{3}H-PrCC binding, also led to a marked decrease (2.3-fold) in the ability of 4-aminopyridine to displace the β-carboline. These results suggest that: (1) the effects of Ni^{2+} on ^{3}H-ligand binding to the brain benzodiazepine receptor appear to be mediated through a Ca^{2+}-associated mechanism; (2) this receptor along with the GABA receptor and the chloride channel seems to be coupled tightly to a putative Ca^{2+} channel; and (3) binding sites for ^{3}H-DZ and ^{3}H-PrCC, which may be subsets of the benzodiazepine receptor, are not associated with this Ca^{2+} channel in the same way.

159.1 ZINC AND GABA BINDING: FROM PATHOLOGY TO PHYSIOLOGY. M. Baraldi. Inst. of Pharmacology, Modena Univ., 41100 Modena, Italy.

We recently demonstrated a pathology of the GABAergic system in hepatic encephalopathy (HE) consequent to fulminant hepatic failure induced by Galactosamine-HCl in rats. The mild stage of HE is characterized by an increase in GABA receptors that are supersensitive to Bicuculline inhibition both in vitro and in vivo, while the severe stage showed a loss of low affinity binding sites. The concomitant decrease in GAD activity allowed us to infer the presence in HE of a denervation supersensitivity phenomenon. In this condition Benzodiazepine receptors are up regulated and supersensitive to GABA stimulation. Since in search of factors responsible of this changes in GABA receptor complex we found a decrease of zinc in brain tissues and a parallel increase in blood, we decided to study this heavy metal as a candidate modulator of GABA binding sites. We present now evidences that zinc is an important factor which can physiologically regulate the binding of GABA to its receptors. It is well known that in fresh synaptic membranes the binding of GABA is very low and that freezing, thawing and washing procedures result in an increase of GABA binding sites by removing endogenous inhibitory factors. Testing the levels of zinc during the above mentioned procedures in synaptic membranes we found a progressive decrease of this metal in the homogenates and a parallel increase in the washing supernatants. The addition of zinc to these membranes leads to a decrease of GABA binding sites at the levels found in fresh no washed membranes (zinc IC_{50} 10^{-5} M). Lineweaver-Burke plot indicates that zinc is a noncompetitive antagonist of GABA receptors. Using frozen, thawed, Triton X-100 treated and extensively washed membranes we got a further decrease in the zinc content of the homogenate which parallels the increase of low affinity binding sites and the unmasking of high affinity GABA receptors (Kd_1=250,Bmx_1=8.5;Kd_2=33, Bmx_2=2). By adding zinc to these membranes we found an inhibition of low affinity GABA binding sites and, with a lesser extent, of the high affinity GABA receptors (Kd_1=220,Bmx_1=4.5;Kd_2=35,Bmx_2= 1.6). It has been demonstrated that factors such as endogenous GABA and peripheral proteins (GABAmodulin) can modulate the GABA receptor unit, however the present findings seem to indicate that other factors can interfere in the regulation of the GABA receptor complex.

159.2 PIPECOLIC ACID- AND GABA SYSTEMS: SIMILARITIES AND DIFFERENCES. E. Giacobini, M.d.C. Gutierrez, H. Nishio* and Y. Nomura*. Lab. of Neuropsychopharmacology, Dept. of Biobehav. Sci., Univ. of Conn., Storrs, CT 06268.

Pipecolic acid (PA) can be synthesized in vitro and in vivo in mammalian brain as a product of lysine metabolism (cf. Giacobini, E., Cell. Mol. Biol., 26: 135, 1980).

Pipecolic acid and GABA mutually inhibit their uptake in mouse brain synaptosomes (Nomura, Y. et al., Neurochem. Res., 5:1163, 1980). However, GABA and nipecotic acid (a powerful non-competitive inhibitor of the uptake of GABA) produce a greater inhibition of synaptosomal uptake of ^{14}C-GABA than of ^{3}H-PA synaptosomal uptake. In addition, PA (10^{-4}M) significantly reduces the synaptosomal and glial uptake of ^{14}C-GABA in the mouse. Pipecolic acid (10^{-4}M) increases K^+-stimulated release of ^{3}H-GABA from rat brain slices, however, GABA (10^{-4}M) affects neither spontaneously nor high K^+ induced release of ^{3}H-PA from rat brain slices (Nomura, Y., Neurochem. Res., 6:391, 1981).

GABA (2.7 mM) and nipecotic acid (2.7 mM) inhibit (30% and 34% respectively) the uptake of ^{3}H-PA (.114 mM) in the mouse brain, following an injection of a double-labeled mixture into the carotid artery (Nishio, H. and Giacobini, E., Neurochem. Res., 6:835, (1981).

The binding of ^{3}H-PA and ^{3}H-GABA to mouse brain fractions and their mutual relation have been studied. Pipecolic acid (5 mM) does not displace ^{3}H-GABA (8 mM) from fresh and frozen membrane preparations.

Our results suggest that GABA and PA are transported and released from and bound to different systems in mouse brain, however, they do not rule out the possibility of a modulatory action of PA on GABA synapses.

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Dr. Y. Nomura and Dr. H. Nishio's present address: Dept. of Pharmacology, Inst. of Pharmaceutical Sci., Hiroshima Univ., School of Med., Hiroshima, Japan.

159.3 GLYCINE AND GABA UPTAKE IN THE MUTANT MOUSE SPASTIC. W. Frost White and Allen H. Heller. Harvard Medical School and Children's Hospital Medical Center, Boston, MA. 02115.

The spastic mutation is a single autosomal recessive mutation that results in hyperexcitability and marked abnormalities in coordinated movement. Electromyographic abnormalities have also been observed, and suggest that these signs result from a defect in spinal cord mechanisms. No anatomical abnormalities have been observed in the CNS of spastic mice. Both the behavioral and electromyographic signs of the spastic mutation can be reproduced in normal mice given subconvulsive doses of strychnine, suggesting that these may be related to a defect in glycine-mediated inhibition in the spinal cord. Biochemical investigations of the postsynaptic glycine receptor, using ^{3}H-strychnine as a ligand, demonstrate at least an 80 % reduction in the binding to spastic spinal cord, brainstem, and midbrain when compared to littermate control animals. In contrast, binding studies for the GABA and benzodiazepine binding sites demonstrate more binding in the spastic spinal cord and brainstem compared to littermate controls. These results support the hypothesis of a defect in glycine-mediated inhibition in spastic animals. They also suggest that the glycine and GABA inhibitory systems interact to maintain inhibitory tone.

In this paper we report our results on the high affinity, Na^+-dependent uptake of glycine and GABA into crude synaptosomal preparations of spastic and littermate control spinal cord and brainstem. In these studies we find 40.4 ± 9.6 % more glycine uptake and 22.5 ± 13.0 % more GABA uptake in spastic than in control animals. Kinetic analyses indicate that these differences result from changes in Vmax and not from changes in Km.

These results indicate that the glycine uptake system is intact in spastic animals. The finding that both glycine and GABA uptake is greater in spastic animals further suggests an interaction in the regulation of the glycine and GABA inhibitory systems in the mammalian CNS. (Supported by NIH grants NS00623, NS11237, and the Children's Hospital Mental Retardation Center Core Grant HD0627.)

159.4 A STUDY OF ^{3}H-GABA UPTAKE BY DISSOCIATED HIPPOCAMPAL CELLS IN CULTURE. J.A. Davidenas* (SPON: C. Rivier). The Salk Institute, La Jolla, CA 92037.

GABA is an important inhibitory transmitter in the hippocampus. Immunycytochemical studies indicate that in vivo it is present in a variety of interneurons, including the so-called basket cells. It is known from physiological studies, that when GABA is iontophoresed onto the somata of hippocampal pyramidal cells in vivo and in vitro, it produces a long-lasting hyperpolarization.

I have examined autoradiographs of hippocampal cells maintained in culture in an effort to determine the number and types of cells which can be specifically labeled with ^{3}H-GABA in vitro. The cells from dissected hippocampi of 18 day rat fetuses were dissociated by mild trypsinization and subsequent trituration in the presence of DNAse. After a brief incubation in a medium supplemented with serum, the cultures were maintained for varying periods in a defined serum-free medium. Cell samples at each time point were preincubated with 1 mM β alanine for 15 minutes prior to incubation with the ^{3}H-GABA to reduce uptake by non-neuronal cells. At different culture ages (between 5 hr and 23 days) the cells were incubated for 30 minutes at 37° C with ^{3}H-GABA (5 x 10^{-8}M or 2 x 10^{-7}M, Spec. act. 28.2 Ci/mmol, New England Nuclear) plus 1 mM β alanine in a buffered salt solution. Immediately after this incubation, the cells were rinsed twice and fixed with a mixture of 1% paraformaldehyde and 1.5% glutaraldehyde, osmicated, dehydrated, and coated with Kodak NTB-2 emulsion. The autoradiographs were exposed for 5 days at 4° C and developed in D19.

The autoradiographs revealed three classes of neurons: (i) The majority of the cells (≈70%) were entirely unlabeled; this class presumably includes most of the hippocampal pyramidal cells. (ii) About 5% of the cells were very heavily labeled. In this morphological category the cells ranged from pyramidal-like neurons to simple bipolar or fusiform cells. (iii) About 25% of the cells were clearly labeled, but not nearly as densely as the preceeding. These cells similarly show considerable variability in form. The distribution of the three classes of cells seems to be established relatively soon after plating, and is maintained, essentially unchanged, from the 4th day in vitro; however, some labeled cells are seen within 12 hours of dissociation.

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159.5 GAMMA-AMINO BUTYRIC ACID BINDING INHIBITION BY HUMAN URINE. J. B. Fiorito*, D. K. Phair*, and A. K. Sinha. Dept. Physiology & Biophysics, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854

Some GABA analogs have been suggested as probable uremic toxins. The inhibitory effect of these analogs on brain gabaergic synapses may explain the etiology of uremic coma. We have investigated the possibility that some normal urinary constituent, other than GABA, might bind to the GABA receptor sites of the brain membranes.

Hamster brain membrane (48,000 g) was frozen, thawed, Triton X-100 treated (37° incubation for 30 min) and washed three times. ^{3}H-GABA binding was performed with ∿ 600 µg membrane protein in the absence of sodium. Morning urine was used from 4 normal, healthy males aged 24-48 yrs. One microliter of urine added to 1 ml incubation volume decreases ^{3}H-GABA binding by 30%. Increasing urine volume to 5, 10, 50, 100 and 200 µl decreases ^{3}H-GABA binding to 40, 29, 10, 5 and 4% respectively. This inhibition is not diminished by removing urinary GABA by gabase treatment. An electrolyte solution simulating human urine was found not to inhibit ^{3}H-GABA binding. The urinary inhibitor is thermostable (boiling water bath for 30 min). The inhibitor is dialyzable (6000-8000 M.W. cut off) and also passes through 500 and 1000 M.W. cut off ultrafiltration membranes. Scatchard analysis reveals that the inhibition is competitive in nature. We have tested the effectiveness of three GABA analogs, present in normal urine, for GABA binding inhibition. Creatine and creatinine have low affinities for the GABA receptor (IC_{50} of 1.3 mM and 3 mM respectively) and guanidoacetic acid has a much higher affinity (IC_{50} = 460 nM).

159.6 Na^+-INDEPENDENT GABA BINDING IN CNS REAGGREGATE CULTURES. J. M. Wehner*, K. Kang* and J. R. Bloomer* (SPON: J. R. Sheppard). Departments of Lab. Med. and Path. and Med., University of Minnesota, Minneapolis, MN 55455.

CNS reaggregate cultures exhibit a variety of differentiated functions including myelination, spontaneous electrical activity and neurotransmitter synthesis and release. Honegger and Richelson (Brain Res. 162:89, 1978) demonstrated that the inhibitory neurotransmitter GABA was released in culture but little is known about the expression of GABA receptors in reaggregate cultures.

Na^+-independent GABA receptors were measured in whole mouse brain reaggregate cultures to determine whether postsynaptic receptors were expressed in culture. Whole brain reaggregate cultures were prepared by the mechanical dissociation method of Honegger and Richelson from timed pregnant Balb/c ByJ mice at 16-17 days in gestation. ^{3}H-GABA receptors were measured at various times in culture using the centrifugation method of Enna and Snyder (Mol. Pharmacol. 13:442, 1977). GABA (10^{-4}M) was used to assess nonspecific binding. Specific saturable Na^+-independent receptors were detectable at all times in culture tested (3-33 days). The average B_{max} was 2.45 ± 0.54 pmol/mg protein. Starting material obtained from 16-17 days gestational animals which underwent mechanical dissociation also had detectable specific binding prior to exposure to culture media. Thus GABA receptors are expressed in late gestational animals, survive the cell dissociation process, and retain their binding characteristics for at least 33 days in culture.

Scatchard analysis of binding data indicated a high affinity ^{3}H-GABA site with a K_d of 15 ± 2.5 nM, which was not altered as a function of time in culture. Solubilization with Triton X-100 detergent was not necessary to reveal this high affinity site. Competition experiments with the agonists muscimol and GABA yielded IC_{50}'s of 2 nM and 10 nM, respectively.

These results indicate that reaggregate cultures not only synthesize and release GABA but also express GABA postsynaptic receptors with characteristics similar to the high affinity GABA receptors of rodent brain. Reaggregates could provide a useful system to examine the effects of drugs or toxic agents on the gabanergic system.

159.7 [^{3}H]-GABA BINDING IN SUBCELLULAR FRACTIONS OF RAT BRAIN. L. Gronke and J.P. Hammerstad, Dept. of Neurology, Oregon Health Sciences Univ., Portland, OR 97201

Most often, pharmacological specificity and regional localization are used to equate ligand binding with neurotransmitter receptors, but subcellular binding patterns can also provide valuable correlations.

We have used analytical differential centrifugation according to the five fraction scheme of deDuve as adapted for brain by P. Lauduron (Int. Rev. Neurobiol. 20:251-281, 1977) to obtain the nuclear (N), heavy mitochondrial (M), light mitochondrial (L), microsomal (P) and supernatant (S) fractions from rat brain. Enzyme activities (lactate dehydrogenase, cytochrome oxidase, β-galactosidase, and Na-K ATPase) were used to monitor the composition of the fractions. Membranes prepared from the homogenate and from the N, M, L and P fractions were (1) dialyzed (without freezing) or (2) frozen/thawed and extensively washed. [^{3}H]-GABA binding was measured by a standard centrifugation assay.

There was no reliable specific [^{3}H]-GABA binding in the N fraction, 35-45% in M, 15% in L and 35-45% in P. Relative specific activity (% of total binding/% of total protein), however, was most enriched in the L and P fraction. Saturation binding experiments showed a single binding site in the L and P fraction but a more complex binding isotherm in the M fraction.

These results are consistent with a localization of the GABA receptor on non-synaptosomal plasma membrane which fragments and distributes mainly into the L and P fractions. The more complex binding in the M fraction (which contains the synaptosomes) could indicate an additional binding site.

159.8 MODULATION OF ^{3}H-GABA AND ^{3}H-MUSCIMOL BINDING BY Ca^{++}. M.G. Corda*, A. Guidotti and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Binding of ^{3}H-muscimol and ^{3}H-GABA was studied in frozen-thawed and repeatedly washed synaptic membranes from rat brain cortices incubated in 20 mM KPO_4 + 50 mM KCl pH 7.0 at 23°C in the presence and absence of Ca^{++}. The addition of Ca^{++} decreased ^{3}H-muscimol binding. The decrease was significant with 0.5 mM Ca^{++} and reached maximal value (about 40%) with 5 mM Ca^{++}. In contrast, the binding of ^{3}H-GABA was increased by Ca^{++}. It has been reported (Nature 290:149, 1981) that Ca^{++} interacts with GABA receptors by revealing a subclass of GABA binding sites ($GABA_B$ binding sites) which can be recognized by baclofen. Therefore the studies of the effect of Ca^{++} on ^{3}H-GABA and ^{3}H-muscimol binding were repeated in presence of $5x10^{-5}$M baclofen, a dose of drug which failed to modify both bindings "per se". Under these conditions Ca^{++} decreased ^{3}H-GABA and ^{3}H-muscimol binding to the same extent. This effect was temperature dependent and was abolished when the membranes were incubated at 0°C. Moreover the effect of Ca^{++} was partially reduced by pretreatment of the membranes with 0.1 mM $AgNO_3$ (Supavilai et al., Neuroch. Internat. 1982, in press) and was reversed by the addition of an excess of EGTA or by washing the membranes at the end of the incubation period. Ca^{++} was more potent than Ba^{++} and Mn^{++}, while Mg^{++} was ineffective at concentrations up to 5 mM. Scatchard plot analysis of ^{3}H-muscimol binding in presence and absence of Ca^{++} revealed that the decrease was due to a reduction in the apparent number of high and low affinity binding sites with no significant change in the apparent dissociation constants.

The inhibition of ^{3}H-muscimol binding by Ca^{++} was not potentiated by the addition of calmodulin (up to 50 µg/ml) and was still present after inhibition of phospholipase A_2 by 1 mM mepacrine or 1 mM tetracaine. Moreover, the effect of Ca^{++} was additive to that of purified GABA-modulin. These data indicate that Ca^{++} decreases the number of $GABA_A$ binding sites while it unveils $GABA_B$ binding sites. The mechanism of these opposite actions is presently under investigation.

159.9 GABA IN CSF AND PLASMA: IMPORTANCE OF CONJUGATED GABA. L. T. Kremzner*, L. J. Côté, J. M. Messing*, F. Kronenberg*, R. Mayeux*, and J. A. Downey* (SPON: J. W. Correll). Departments Rehabilitation Medicine and Neurology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

GABA (γ-aminobutyric acid), a major inhibitory neurotransmitter, occurs at micromole/gram levels in brain and spinal cord and at picomole/ml. levels in the cerebrospinal fluid (CSF). To delineate the relationship of GABA (free and conjugated) in different physiological pools we determined GABA in human CSF and plasma using HPLC coupled with O-phthalaldehyde fluorescent measurements. CSF and blood were placed on ice immediately after collection and rapidly processed at 4°C. Free and conjugated GABA were extracted with perchloric acid (PCA); conjugated GABA was determined on the PCA extract after 6N HCl hydrolysis at 110°C for 24 hr. Using these procedures, free GABA in lumbar CSF, obtained from patients with neurological disorders, ranged from 50 to 500 pmoles/ml; ventricular fluid GABA levels are 10 to 20 fold greater. After acid hydrolysis, the GABA values increased to between 3,000 to 6,000 pmoles/ml, a 15 to 100 fold increase. The increase in CSF GABA could not be accounted for by the hydrolysis of homocarnosine (γ-aminobutyrylhistidine) indicating that other GABA conjugates are also present. Unconjugated (free) plasma GABA levels varied from 300 to 1500 pmoles/ml; conjugated GABA levels varied from 1,500 to 7,300 pmoles/ml. The origin of CSF GABA is presumed to be from the central nervous system (CNS), while the origin of plasma GABA is uncertain. Because of concentration and volume differences, total plasma GABA is up to 10 fold greater than total CSF GABA. Preliminary studies indicate that under circumstances of elevated unconjugated plasma GABA, there is a concomitant but lesser increase in conjugated GABA, suggesting a dynamic relationship between the two GABA forms. Studies are in progress to characterize the chemical nature of the GABA conjugates in CSF and plasma.

159.10 CROSS TOLERANCE TO THE ANTINOCICEPTIVE EFFECTS OF MORPHINE AND THIP, A GABA RECEPTOR AGONIST. T.H. Andree* and S.J. Enna, Depts. of Pharmacology and of Neurobiology and Anatomy, Univ. Texas Medical School, Houston, TX 77025.

THIP (4,5,6,7-tetrahydroisoxazolo(5,4-c)-pyridin-3-ol) is a direct acting GABA receptor agonist which has been shown to possess antinociceptive potential in laboratory animals and man. At a dose of 7.5 - 10 mg/kg (i.p.) in mice THIP produces maximal analgesia which can be antagonized by atropine but not by naloxone. These data suggested that THIP analgesia is not mediated through an action on the brain opiate system (J. Pharmacol. Exp. Therap. 220:482, 1982). To determine whether tolerance develops to the antinociceptive effect of THIP, mice were treated for five days (7.5 mg/kg, i.p., b.i.d.) and analgesia tested on the sixth day using both tail immersion and hot plate methods. In both cases the dose-response curves for THIP indicated a significant reduction in the potency of this compound in the chronically treated animals. Moreover, some tolerance to the analgesic effect of morphine was also noted in these animals. Additional experiments revealed that morphine-dependent mice were tolerant to the analgesic effects of both THIP and morphine. Preliminary data indicate no changes in brain receptor binding for either GABA or the cholinergic muscarinic systems in the THIP tolerant animals. These data suggest that the opiates and GABA agonists act through separate, but related, pathways to induce an analgesic response. Thus, THIP and related GABAmimetics may be useful substitutes for opiates in the treatment of pain.

159.11 GAMMA-AMINOBUTYRIC ACID ALTERS THE PO_2 DISTRIBUTION IN INTACT GERBIL BRAIN. E.K. Angelopoulos*, P.E. Coyer, & L.J. Bearden (SPON: G.V. Pegram). Dept. Neurology, Neurosciences Program, & Dept. Biomedical Engineering, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Several findings have suggested a specific association of gamma-aminobutyric acid (GABA) with cerebral blood vessels. The enzymes for both the synthesis and degradation of GABA have been found in elevated concentrations around cerebral vessels. It has also been observed that elevated CSF concentrations of GABA occur in humans during pathological conditions such as migraine headache and cerebral anoxia. These increased levels correspond to the concentration of GABA which has been shown to effectively induce the relaxation of isolated cerebral arteries in vitro. Additional pharmacological studies have verified the presence of GABA receptors on the wall of cerebral arteries (Edvinsson & Krause, Brain Res. 173: 89-97, 1979; Krause, et al., Brain Res. 185: 51-57, 1980). Therefore, there is substantial in vitro evidence which suggests a role for GABA in the control of the cerebral circulation.

Using a PO_2 microelectrode and adjacent pressure microinjection pipette, unit activity (action potentials) and PO_2 were recorded from the brains of anesthetized gerbils (held at 37°C) before, during, and after GABA injection. Bipolar EEG was also monitored. Following microinjection of GABA, the time to reach the maximum response in PO_2 varied from 25-40 sec, but in each case there was an increase in the PO_2 above the control (resting) level. In some cases these responses lasted several minutes. Using phosphate buffer loaded micropipettes (pH=7.2) no increases in the brain PO_2 were observed in control experiments. The duration of the GABA-mediated increase in PO_2 lasted several minutes depending upon the concentration of injected GABA and the length of the injection pulse. A transient increase in PO_2 with GABA pressure microinjection followed by a temporary cessation of spontaneously-firing units was also observed. Return of neural activity occurred as the PO_2 continued to increase. These results suggest a possible vasodilatory effect of GABA on the cerebral circulation as well as an alteration of neuronal activity.

159.12 INTRACELLULAR ANALYSIS OF GABA-EVOKED RESPONSES OF FROG PRIMARY AFFERENT AXONS. A.L. Padjen and T. Hashiguchi, Dept. of Pharmacology & Therapeutics, McGill University, Montreal, PQ H3G 1Y6

Previous studies, using sucrose gap recording on dorsal roots in frog spinal cord, indicated the existence of two types of GABA responses on primary afferents: a bicuculline-sensitive depolarization and a bicuculline-resistant hyperpolarization (Bourne & Padjen, Neurosci. Abst. 6: 148, 1980). Present experiments were initiated to examine sensitivity to GABA of primary afferent axons using intrafiber recording (KCl filled microelectrodes) at the root entry of isolated hemisected frog spinal cord preparation, continuously perfused with Ringer at 10°C (Hashiguchi & Padjen, Neurosci. Abst. 7: 437, 1981). Successful recording was obtained from over 40 large myelinated axons (resting membrane potential >70 mV; conduction velocity >15 m/s). Perfusion with GABA (0.1 - 5 mM) in normal Ringer depolarized the majority of fibers with a prominent fading of response. In 5 fibers GABA caused a hyperpolarization (up to 6 mV), accompanied by disappearance of spontaneous activity, shortening of duration of primary afferent depolarization and no change or increase in effective resistance (Re). After blockade of synaptic transmission GABA depolarized all fibers (4 mV and 10 mV, for 0.2 and 1 mM, respectively; no difference between Mn and TTX blockade). These depolarizations were characterized by a fast rise and were accompanied by a net decrease in Re. Fading of the plateau was prominent with higher conc of GABA. The equilibrium potential, obtained by extrapolation, was -30 mV. The GABA analog kojic amine (KJA; 0.1 - 1 mM) caused a slowly developing depolarization without fading (amplitude comparable to GABA response), but was present in only 9 of 30 fibers tested. Baclofen (0.1 mM) caused a small (<2 mV) hyperpolarization (in 2/7 fibers) without any detectable change in Re or propagating action potential. Bicuculline (0.1 mM) reversibly antagonized depolarizing responses to GABA and KJA without direct effect on electrical properties of fibers. In one fiber, the depolarizing response to KJA was reversed by bicuculline to hyperpolarization. These results suggest that: 1) the GABA-evoked hyperpolarization recorded in normal Ringer is indirect resulting from inhibition of tonic and evoked interneuronal activity. 2) Direct hyperpolarizing responses to GABA, KJA and baclofen, seen in sucrose gap recording, may not be present in large myelinated fibers. 3) KJA may activate a separate bicuculline sensitive (possibly GABA-) receptor not present in all primary afferent fibers.

Supported by MRC.

159.13 USE OF GABACULINE INDUCED GABA ACCUMULATION FOR AN INDEX OF RETINAL GABA SYNTHESIS. M. A. Proll* and W. W. Morgan. Dept. of Anatomy, The Univ. of Texas Health Science Center, San Antonio, TX 78284.

GABA is thought to be a major inhibitory neurotransmitter in the central nervous system, but, until recently, there was a limited number of techniques available for measuring GABA synthesis. With the advent of relatively specific GABA transaminase (GABA-T) inhibitors, GABA accumulation following the inhibition of the degradative enzyme GABA-T can be used as an index of GABA synthesis (Bernasconi, et al., J. Neurochem. 38: 57, 1982). We decided to see if this method could be used as an index of GABA synthesis in the rat retina. Gabaculine was injected intravitreally, and unless specified otherwise, all experiments were performed on male Sprague-Dawley rats under ordinary laboratory fluorescent lighting. GABA was measured by gas-liquid chromatography. The accumulation of GABA after gabaculine was both dosage- and time-dependent. Dosages of 0.05 µg, 0.10 µg and 0.25 µg of gabaculine per eye injected at 60 min prior to sacrifice all significantly increased retinal GABA content, but the increase measured with 0.05 µg was significantly less than the accumulation with the 2 higher dosages. The accumulation with 0.10 and 0.25 µg gabaculine/eye did not differ, thus the 0.10 µg/eye dosage was used in subsequent experiments. Next GABA was measured at 0, 30, 60 and 90 min after gabaculine treatment in both light- and dark-adapted rats. In the dark-adapted experiment, rats were dark-adapted for approximately 15 hours, and the experiment was then performed in a photographic darkroom with light provided by a photographic red safe light. In both groups, the linear increase in retinal GABA content over the 90 min treatment period was approximately 3 fold, and the rates of GABA accumulation in the dark- (0.054 µg GABA/min/retina) and light- (0.051 µg GABA/min/retina) adapted groups were similar. The GABA agonist muscimol (MUS) and the GABA antagonist bicuculline methiodide (BIC) significantly suppressed and facilitated, respectively, the gabaculine induced increase in GABA levels. All dosages of MUS (0.375, 1.5 and 3.0 nmoles/eye) significantly depressed gabaculine-induced GABA accumulation, and the effect was dosage-dependent. The antagonist BIC was able to partially block the inhibitory effect of MUS. Gabaculine-induced GABA accumulation appears to be a useful technique for measuring the rate of GABA synthesis in the retina. Supported by DA00755 and RSDA DA00083-06A1 to WWM.

159.14 FLURAZEPAM INHIBITS THE GABACULINE INDUCED INCREASE IN GABA CONTENT IN THE RETINA. S. L. Lane* and W. W. Morgan (SPON: A. Miller). Dept. of Anatomy, The Univ. of Texas Health Sci. Ctr. at San Antonio, TX 78284.

Benzodiazepines (BZ) in the CNS are believed to exert their action by potentiating GABAergic transmission. BZ receptors have also been demonstrated in the retina but their physiological significance remains to be elucidated. It has been recently shown in our laboratory that flurazepam (FLU), a water soluble BZ, exerts an inhibitory action on dopamine (DA) neuron activity although this does not appear to be mediated through the GABA system (Kamp and Morgan, Eur. J. Pharmacol. 77: 343, 1982). Therefore, the purpose of the present experiments was to determine if the BZ exert any influence on the retinal GABAergic neurons. In the brain, BZ have been shown to suppress GABA synthesis (Bernasconi, et al, J. Neurochem. 38: 57, 1982).

Adult male albino rats were given intravitreal injections of saline, gabaculine, a GABA-T inhibitor (.1 µg/eyeball), varying doses of FLU (0.125µmole, 0.25 µmole, 0.5 µmole or 1.0 µmole/retina), or varying doses of FLU plus the single dose of gabaculine. The animals were sacrificed one hour later. The administration of gabaculine alone resulted in a marked enhancement of GABA content over saline controls ($p<.01$). FLU alone had no effect on GABA content. However, when combined with gabaculine, FLU prevented the gabaculine induced increase in GABA content in a dose-dependent manner.

These results lend support to the hypothesis that the BZ may influence the GABA system via a negative feedback loop. FLU may act to potentiate GABA action at post-synaptic receptor sites. The post-synaptic interpretation of this potentiation would be that there was an increase in available GABA. This could trigger a long loop negative feedback system which would effectively inhibit further synthesis of GABA. Alternatively FLU could be potentiating an autoreceptor system wherein GABA feedback inhibits its own synthesis. Additional investigations are required to differentiate between these possibilities. Supported by DA00755 and RSDA DA00083-06A1 to WWM.

159.15 BACLOFEN: DEPRESSION OF NEURONAL FIRING IN THE HIPPOCAMPAL SLICE. Brian Ault* and J. Victor Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

In the rat hippocampal slice the anti-spastic drug, baclofen, preferentially depresses transmission at synapses made by axons of CA3 pyramidal cells. Bicuculline (BIC) cannot reverse this effect of baclofen. We have suggested that baclofen acts at a BIC-insensitive GABA receptor located on one type of glutamatergic/aspartergic synaptic terminal to depress the release of excitatory transmitter. In the present study, we examined whether, in addition, baclofen acts postsynaptically to reduce the firing of hippocampal neurons.

(±)Baclofen (20 µM) completely suppressed the synchronous firing of CA1 pyramidal cells in response to stimulation of stratum radiatum or stratum oriens and of CA3 pyramidal cells in response to stimulation of stratum radiatum. In contrast, the firing of CA3 pyramidal cells evoked by mossy fiber stimulation was unaffected and the firing of dentate granule cells evoked by perforant path or associational-commissural stimulation was reduced by only 43% and 21%, respectively. When tested on the population spike evoked by stimulation of the Schaffer collateral-commissural fibers in area CA1, (-)baclofen depressed the population spike with an IC_{50} of about 2 µM. (+)Baclofen was about two orders of magnitude less potent. Input-output curves, in which population spike amplitude was plotted against the amplitude of the corresponding Schaffer collateral-commissural extracellular EPSP, were unaffected by baclofen. In contrast, imidazole-4-acetic acid (IAA) and 3-aminopropanesulfonic acid (3-APS), which are thought to act largely at postsynaptic GABA receptors, reduced the slope of the input-output curve. The depressant effect of (-)baclofen on the population spike was little affected by 100 µM BIC, but depressions produced by 3-APS and IAA were readily antagonized. GABA-induced depressions were much less sensitive to BIC than those produced by the latter two analogues. These results are consistent with the effects of baclofen and other GABA agonists on extracellular EPSP's in the hippocampal slice and suggest that the baclofen-induced depression of synaptically-evoked firing can be explained by an interaction with presynaptic BIC-insensitive GABA receptors.

(-)Baclofen at submicromolar concentrations shortened the period of burst firing evoked in area CA1 by antidromic stimulation in the presence of BIC. This action appears to be postsynaptically mediated. These observations suggest that agonists at BIC-insensitive GABA receptors might be useful limbic anticonvulsants. (Supported by NIH grant NS 16064.)

159.16 RELEASE OF ENDOGENOUS, OF ENDOGENOUSLY FORMED LABELLED AND EXOGENOUS LABELLED GABA BY HIGH K^+ OR VERATRIDINE IN RAT HIPPOCAMPAL SLICES. J. C. Szerb. Department of Physiol. Biophysics, Dalhousie U., Halifax, N.S., Canada, B3H 4H7

To see how the release of GABA freshly formed from glutamate (glu) compares with that of endogenous (endo) unlabelled or exogenous (exo) labelled GABA, rat hippocampal slices were incubated for 30 min in 3 mM K^+ with 5 µCi/ml [3,4-^{3}H]l-glutamate and 0.5µ Ci/ml [U-^{14}C] GABA and were superfused first with 3 mM K^+ then for 36 min with either 50 mM K^+ or 50 µM veratridine (V) in the presence or absence of Ca^{2+}. Endo GABA was measured by HPLC which was also used to separate labelled GABA from labelled glu and labelled metabolites. Although 10 times less [^{14}C] GABA than [^{3}H] glu was present in the incubation fluid, slices contained twice as much [^{14}C] GABA than [^{3}H] GABA 15 min after labelling. During the following 48 min perfusion with 3 mM K^+ the content of [^{14}C] GABA but not that of [^{3}H] GABA declined. In the presence of Ca^{2+} 50 mM K^+ released [^{14}C] GABA with a higher specific activity (SA) than that in the slices while the SA of released [^{3}H] GABA was about equal to that in slices. Exposure for 36 min to V in the presence of Ca^{2+} caused an initial large release of both endo and labelled GABA followed by a rapid decline. The SA of [^{14}C] and [^{3}H] GABA released were the same as with 50 mM K^+. In the absence of Ca^{2+} release induced by 50 mM K^+ was much reduced but that evoked by V was increased. Depolarization with 50 mM K^+ in the presence of Ca^{2+} induced a synthesis of endo GABA in excess of release but V, in the presence or absence of Ca^{2+}, or 50 mM K^+, in the absence of Ca^{2+}, failed to induce excess GABA synthesis. Results suggest: (1) Exo labelled GABA is preferentially taken up into releasable stores while GABA synthesized from exo glu is distributed uniformly. (2) Considerable Ca^{2+}-independent release of GABA induced by V in the absence of a GABA-T inhibitor originates from neurons since GABA taken up by glia would have been metabolized. (3) An increase in extracellular K^+ in the presence of Ca^{2+} rather than depolarization per se may regulate GABA synthesis.

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159.17 COMPOUND LY81067, A DIARYLTRIAZINE, AUGMENTS GABA-MEDIATED INHIBITION IN RAT HIPPOCAMPUS. B. S. Lamishaw*, W. B. Lacefield* and R. C. A. Frederickson. Lilly Research Labs., Eli Lilly and Company, Indianapolis, IN 46285.

Biochemical and electrophysiological evidence suggests some form of coupling between the action of γ-aminobutyric acid (GABA) and benzodiazepines (BZD). GABA enhances BZD binding to neuronal membranes and under certain conditions diazepam (DZ) increases GABA binding. A novel diaryltriazine compound, LY81067, has been found to enhance the binding of ^{3}H-flunitrazepam and ^{3}H-GABA to neuronal receptors, without itself binding to these receptors. We have utilized an electrophysiological model to distinguish GABA-like and BZD-like effects in order to characterize the properties of this and related compounds. Rats were anesthetized with urethane and recording and stimulating electrodes were placed in the CA1 area of hippocampus and contralateral hippocampus, respectively, using stereotaxic techniques. This allowed orthodromic activation of pyramidal cells in CA1 and recording of the resulting population spike. A paired-pulse paradigm was utilized in which the second population spike is reduced in amplitude due to GABA-mediated recurrent inhibition.

Bicuculline (0.1 mg/kg i.v.), but not strychnine (0.7 mg/kg i.v.), antagonized the recurrent inhibition of the second spike confirming mediation by GABA. GABA-like agents such as muscimol (5 mg/kg i.v.), THIP (5 mg/kg i.v.) and Progabide (10 mg/kg i.v.) reduced the amplitude of both population spikes. DZ (0.1 mg/kg i.v.) and LY81067 (0.5 mg/kg i.v.) preferentially inhibited the second spike. Doses ten times higher did not affect the first spike. Thus DZ and LY81067 appear to selectively potentiate GABA-mediated recurrent inhibition. Preliminary experiments suggested that β-carboline-3-carboxylic acid ethyl ester (6 mg/kg i.v.) antagonizes the effects of DZ and LY81067, but not that of muscimol. Thus LY81067 is unique in potentiating GABA action in a manner electrophysiologically similar to that of DZ, but without binding to either GABA or BZD receptors.

159.18 COMPOUND LY81067, A DIARYLTRIAZINE, ENHANCES BENZODIAZEPINE AND γ-AMINOBUTYRIC ACID BINDING TO RAT BRAIN RECEPTORS. Frank P. Bymaster*, William B. Lacefield* and David T. Wong. Lilly Res. Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Compound LY81067, enhanced the receptor binding of ^{3}H-flunitrazepam (^{3}H-FLU) and ^{3}H-γ-aminobutyric acid (^{3}H-GABA) in cerebral cortical membranes. Stimulation of 50 percent ^{3}H-ligand binding above control occurred at LY81067 concentrations (SC_{50}) of 700 and 250 nM, respectively. The maximal stimulation on binding of both ^{3}H-ligands was higher and the SC_{50} lower for LY80167 than the pyrazolopyridines, tracazolate, etazolate and cartazolate. The saturation binding of ^{3}H-FLU (0.2 to 10 nM) was increased at each concentration by LY81067; Scatchard analysis revealed that predominately the affinity constant (Kd) was decreased from 1.44±0.04 to 0.80±0.03nM and the number of binding sites was increased from 1247.4±21.7 to 1500.6±32.6 fmoles/mg protein. The Hill coefficient was 0.95±0.02 for control and 0.93±0.02 for LY81067-treated membranes. The saturation binding of ^{3}H-GABA (0.5-60nM) was increased at each concentration by LY81067; Scatchard analysis revealed that ^{3}H-GABA bound to 2 components of sites, both of which were stimulated by LY81067. The Kd for each component was decreased and the number of binding sites was increased. The binding of ^{3}H-GABA and ^{3}H-FLU to receptors from cerebral cortex, cerebellum, pons-medulla, hippocampus, corpus striatum, and midbrain were all enhanced by LY81067. The GABA antagonists, picrotoxin and bicuculline, partially antagonized the enhancement of ^{3}H-FLU binding by LY81067. Bicuculline at 10μM changed the SC_{50} of LY81067 from 470nM to 6000nM. The SC_{50} for LY81067 enhancement of ^{3}H-FLU binding was lowered from 6800 to 800nM by addition of chloride anion to a chloride-free medium, suggesting involvement with the chloride ionophore. Treatment of membranes with the detergent Triton x-100 decreased the ability of LY81067 to enhance the binding of either ligand; however, the specific binding of the ligands was not decreased by this treatment and GABA was able to enhance ^{3}H-FLU binding in these membranes. This suggests the solubilization of a component(s) which is required by LY81067 to stimulate binding and that this mechanism is different from that required for GABA-induced elevation of ^{3}H-FLU binding.

159.19 MUSCIMOL-SCOPOLAMINE INTERACTIONS IN THE RAT BRAIN: A STUDY WITH [14-C]2-DEOXY-D-GLUCOSE. P. Helén* and E. D. London. (SPON: G. A. Young) Lab. of Neurosci., Natl. Inst. on Aging, Gerontology Res. Ctr., Baltimore City Hosp., Balto., MD 21224.

The [14-C]2-deoxy-D-glucose ([14-C]DG) method of Sokoloff et al. (J. Neurochem. 28:897,1977) has been used to show that γ-aminobutyric acid (GABA) agonists (muscimol and 4,5,6,7-tetrahydroisoxazolo-[5,4-C-]-pyridin-3-ol, THIP) produce dose-dependent decreases in local cerebral glucose utilization (LCGU) of many brain regions (Palacios, J.M. et al., Eur. J. Pharmacol. 71:333, 1981). Because of evidence for cholinergic components in some actions of GABA-mimetic drugs, we extended our previous observations on GABAergic effects on LCGU, and assessed the cholinergic involvement in GABA-mimetic action by measuring LCGU in rats treated with muscimol in the presence or absence of scopolamine, a muscarinic antagonist.

250-350 g male Osborne-Mendel rats were used in this study. Four hr after surgical insertion of cannulae into the right femoral artery and vein, each rat was given one of the following treatments: saline (1 mg/kg, 30 min and 14 min before [14-C]DG), muscimol (4 mg/kg, 16 min after saline, 1 ml/kg, and 14 min before [14-C]DG), scopolamine (0.4 mg/kg, 30 min before [14-C]DG and 16 min before saline, 1 ml/kg), scopolamine (0.4 mg/kg, 30 min before [14-C]DG) + muscimol (4 mg/kg, 14 min before [14-C]DG). All injections were given i.v.. LCGU was determined as described by Sokoloff et al. (1977).

Muscimol significantly ($p \leq 0.05$) reduced LCGU in the following brain regions: medial geniculate body (35%), nucleus accumbens (26%), auditory cortex (48%), frontal cortex (52%), and thalamic nuclei [ventral (38%), ventromedial (37%), reticular (25%), reuniens (23%) posterior (39%) and parafascicular (26%)]. 0.4 mg/kg scopolamine did not antagonize the effects of muscimol in any brain region. Scopolamine alone reduced LCGU in the media geniculate body (23%), auditory cortex (28%) and frontal cortex (24%). LCGU reductions in response to scopolamine + muscimol were greater than after muscimol alone in the anterior thalamus (45% vs. 21%), periventricular nucleus of the thalamus (45% vs. 21%) and medial cortex (51% vs. 26%). In the following regions, where neither muscimol nor scopolamine decreased LCGU, significant decrements were seen after combined treatment with both drugs: olfactory bulb (49%), lateral habenula (19%) and dentate gyrus (26%).

These findings support the concept of GABAergic-cholinergic interactions in the rat brain and suggest that some GABA-mimetic effects can be enhanced by anticholinergic drugs. Further studies with extended dosage ranges of GABA-mimetic, cholinomimetic and cholinolytic drugs are required for a more complete eluciation of these interactions.

159.20 CHANGES IN 2DG BRAIN METABOLISM INDUCED BY DIRECT GABA AGONISTS. G.T. Golden, R.G. Fariello, G. Alexander* and R.J. Schwartzman. Medical Service, Neurology Section, VA Hospital and University of Texas Health Science Center at San Antonio, Texas 78284.

The direct GABA agonists muscimol (5-Aminomethyl-3-hydroxyisoxazole) and THIP (4,5,6,7 tetrahydroisoxazole (5, 4-C pyridin) 3-oL) have been proposed for clinical application in disease states demonstrating possible impaired GABA mediated inhibition (such as extrapyramidal dysfunctions, pain, psychosis, and seizures). We have examined patterns of metabolic activity in the brains of rats, using the quantitative 14C-2-deoxy-o-glucose technique, after a single i.v. administration of either muscimol (0.7 mg/kg) or THIP (10.0 mg/kg). Rats were anesthetized with ketamine HCl and N20. Cannulas were inserted in the femoral vein and artery. EEG leads were connected to the scalp and the animal was placed in a restrainer and allowed to recover. After recovery, a baseline EEG was recorded for 20 minutes. Ten to twenty minutes after administering a direct GABA agonist, a bolus of 14C 2DG (7.5 μci/100g) was injected via the femoral vein catheter. Arterial blood samples (0.15cc) were obtained at 15 sec intervals for the first minute, 2,4,6,10 minutes, and every 5 minutes for the remainder of the study (45 minutes after the initial bolus injection), for the determination of 14C concentration. Forty-five minutes after 2DG the animals were sacrificed, brains removed, frozen in liquid nitrogen, and cut in 20μ sections; these were placed on microscopic slides and exposed along with plastic 14C standards on Kodak SB-5 x-ray film for 14 days. The 14C concentration of each structure was computed from its optical density as compared to that of the standards. The local cerebral metabolic rate for glucose lCMRg was calculated quantitatively according to its method of Sokoloff (J Neurochem 28:897-916, 1977). The mean lCMRg from the experimental animals were compared to controls by 1 way ANOVA.

Immediately after injection of muscimol, the EEG showed paroxysmal bursts of 4-6 c/s; spike and wave lasting through the experiment. Sustained EEG changes occurred within 30 seconds after THIP, spikes, polyspikes and spike wave occurred bilaterally with a tendency to become rhythmic. 2DG analysis revealed a remarkable alteration of brain metabolic activity compared to control animals. Several neural areas in the experimented animals demonstrated similar patterns of metabolic activity. These include the lateral habenular nucleus, nucleus accumbens, medial septal nucleus, superior olivary complex and the inferior colliculus. All the aforementioned structures showed an increase in lCMRg after both THIP and muscimol. The only common neural area showing a decrease in lCMRg for both muscimol and THIP groups was the motor cortex. In addition, several neural areas showed changes in lCMRg specific to the particular GABA agonist administered.

159.21 POTENTIAL DIAGNOSTIC VALUE OF PLATELET GABA-TRANSAMINASE AND PLASMA GABA IN AFFECTIVE ILLNESS. B. Umberkoman-Wiita, W.H. Berrettini*, T.A. Hare and W.H. Vogel. Department of Pharmacology, Thomas Jefferson University, Philadelphia, Pennsylvania and Section on Psychogenetics, National Institute of Mental Health, Bethesda, Maryland.

Recent studies (Gerner and Hare, 1981; Gold et al., 1980) have demonstrated low CSF GABA in depressed patients. To determine whether the peripheral GABA system was affected in the same way, we measured plasma GABA and platelet GABA-transaminase activity in euthymic medication-free bipolar patients and in normal volunteers. The bipolar group (n = 9) showed significantly lower platelet GABA-transaminase activity ($p < .035$, two-tailed "t" test) and lower plasma GABA ($p < .02$). Additionally, twin studies revealed that both platelet GABA-transaminase activity ($r_i = .57$, $p = .035$) and plasma GABA levels ($r_i = .72$, $p = .007$) were concordant in monozygotic twin pairs (n = 9). We found no correlation between platelet GABA-T and plasma GABA, indicating that platelet GABA-T levels may not play a part in limiting circulating GABA levels. These studies suggest that plasma GABA and/or platelet GABA-transaminase may be genetic markers for vulnerability to affective illness.

159.22 GLYCINE, NEUROACTIVE AGENT EVALUATION. A. de la Sierra, O. González*, V. Santoni*, A. Reyes*, University of P. R., Cayey Campus 00634.

The effect of glycine & its carboxy-borane derivative on the force and rate of contraction of intestinal, cardiac and skeletal muscle of Bufo marinus was registered in a 564B Tektronic oscilloscope with memory storage from a force transducer. Central synaptic effects were also measured by registering force and rate of contraction as well as EMG of gastrocnemius contralateral to a site of sciatic stimulation. All observations were made during a 10 min. period after treatment with 1.0 mg of glycine, epinephrine and a.choline. The interactions of these drugs were also evaluated. Immediately after adding glycine to a 2.0 cm segment of small intestine submerged in 35 cc of aerated glucose-amphibian Ringer's, there was a relaxation of 500 mg of tension sustained for variable periods. Epinephrine caused a dramatic increase in tension of 1.25 grams when compared to only 375 mg from a.choline. Rate of contraction of smooth muscle was too slow and variable to quantitate. This effect was not inhibited by atropine. Alpha and Beta blockers are being evaluated in this respect. The effect of glycine on miocardium was similar to that of a.choline when applied over intact heart of decerebrated frog. Soon after glycine treatment there was a relaxation of 500 mg and a bradycardia of 30/min. responsible for an increased diastolic filling that periodically provoqued a violent Sterling contraction of 750 mg during sistolic ejection. The arrhytmia persisted for 6 min. Epinephrine had variable effects. The central synaptic results are inconclusive and the experimental design subject to re-evaluation. With the aid of the MNDO computer program (Univ. of Indiana), we are making theoretical evaluations of these results as they relate to conformational changes in glycine and their Boron derivatives, currently assayed.

159.23 TAURINE PRODUCES GABA-LIKE INHIBITION OF EVOKED POTENTIALS IN CAT SOMATOSENSORY CORTEX S. Brailowsky and R. T. Knight*, Clinical Neurophysiology Laboratory, U.C. Davis, V.A. Medical Center, Martinez, CA 94553.

The primary SEP in the cat consists of a surface positive followed by a surface negative deflection. Previous studies suggests that the positivity reflects depolarization of cell bodies of neurons in layer IV and that the aftergoing negativity reflects depolarization of corresponding apical dendrites. GABA has been shown to inhibit this aftergoing negativity of the primary SEP in the cat. Taurine produces GABA-like inhibition in several physiological systems. We investigated the effects of acute direct cortical application of low doses (1-100 ug/ul) of GABA and taurine on the cat primary SEP. Twenty animals were studied in acute preparation under barbiturate anesthesia with monitoring of core temperature and controlled expired carbon dioxide concentration. The cortical surface was maintained by warmed saline drip. Both GABA and taurine produced rapid, equivalent and reversible inhibition of the negativity of the primary SEP. Saline control and glutamate had no effect. We were unable to inhibit the taurine and GABA effects by pre-treatment with bicuculline or picrotoxin, even when these substances induced epileptogenic activity at the application site.

We conclude that even if taurine is not considered a true neurotransmitter, its physiological effects on superficial layers of the somatosensory cortex are indistinguishable from those of GABA.

160.1 CHARACTERIZATION OF VASOPRESSIN-STIMULATED ORNITHINE DECARBOXYLASE IN MOUSE BRAIN. R. Tintner, H.E. Gray, W.G. Luttge and A.J. Dunn. Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

The polyamines are believed to modulate cellular responses to a number of hormones and neurosecreted agents. Ornithine decarboxylase (ODC) is the initial, and probably rate-limiting, enzyme in their biosynthesis. We earlier showed that intracerebroventricular (icv) injections of either lysine-vasopressin (LVP) or saline-increased ODC activity in adult mouse brain, but with different time courses. The effect of LVP, however, was much larger and dose-dependent (Tintner et al., Trans. Am. Soc. Neurochem., 12:150, 1981). The present studies extend the endocrine and enzymologic characterization of this effect.

Male CD-1 mice (25-32 gm) were used. All peptides were injected bilaterally icv in a total of 2 ul and animals sacrificed 3h after injection. ODC was assayed routinely by capture of $^{14}CO_2$ from 1-[^{14}C]-L-ornithine substrate. LVP, ACTH, PRO-LEU-GLY-NH_2 (PLG), desamino-8-arginine-vasopressin (DDAVP) or oxytocin injections increased brain ODC activity 206, 190, 160, 128 and 102% of saline control, respectively. Adrenalectomy prevented the increase in ODC activity by LVP relative to saline but not the smaller increase seen in injected relative to uninjected animals. High dose dexamethasone restored the former but suppressed the latter effect. The enzyme activity was localized exclusively to the cytosol fraction by the routine assay with added γ-acetylenic GABA (Dow-Merrell) and by assay of ^{14}C-putrescine from U-[^{14}C]-L-ornithine. Cytosol from LVP-injected adult and uninjected neonatal mouse brain was subjected to Sephacryl (S-300) gel filtration and ultracentrifugation on 10-30% glycerol linear gradients. The adult and neonate preparations behaved identically with the following hydrodynamic parameters: $S_{20,\omega}$ 3.8±.1 X 10^{-13} s^{-1}, Stokes' radius 38.5±1.6 X 10^{-8} cm, molecular weight 63±4 X 10^3 Daltons, f/f_o 1.5.

These data are discussed in relation to the known behavioral and biochemical effects of vasopressin and its analogs as well as the physical characteristics of ODC from other tissues.

160.2 MODULATORY EFFECTS OF CATECHOLAMINES AND VASOPRESSIN ON NEURONS IN THE LATERAL SEPTUM. J.E. Marchand and N. Hagino. Dept. of Anatomy, Univ. TX Health Sci. Ctr. at San Antonio, TX 78284

Neurons containing Dopamine (DA), norepinephrine (NE), and vasopressin are known to project to the lateral septum; furthermore, these neurotransmitters have been shown to be involved in functions controlled by the septal-hippocampal axis. In this study, we have used electrophysiological and iontophoretic techniques to examine the effects of these agents on responses of lateral septal neurons driven by stimulation of hippocampal efferents.

Field and extracellular unitary potentials were recorded (using glass pipette containing .2M NaAcetate and Pontamine Sky Blue) from neurons in the lateral septum of urethane anesthetized (1 g/Kg bwt) Sprague-Dawley albino rats. Stimulation of the fimbria-fornix, using bipolar stainless steel electrodes (250 µm o.d., 300 µm intertip distance) evoked two prominent negative field potentials in the lateral septum. The early negativity developed a peak amplitude at 4-6 msec, and latencies of unitary spikes driven by stimulation corresponded with the field negativity; units were activated at latencies of 4-7 msec and appeared to be monosynaptically driven, as determined by small latency shifts during decreased intensity of stimulation. The later polysynaptic negative field potential (peak amplitude 10-12 msec) with associated unitary spikes (9-13 msec latencies) was also activated by fimbria-fornix stimulation, and was particularly prominent in the ventral portion of the lateral septum. Further, it was found that decreasing the stimulus intensity increased the size of the late negative field, and caused an increased probability of firing of the septal unit at the late vs the early latency. DA, NE, and arginine-vasopressin (AVP) were iontophoresed upon these septal units, and it was found that each of these agents reduced the probability of the unit firing at the first latency, while increasing the probability of firing at the second latency. These data suggest that the function of these transmitters might be to enhance polysynaptic responses over monosynaptic responses. (Supported by NICHD-HD-10071.)

160.3 NEUROTENSIN INCREASES DOPAMINE TURNOVER IN RAT BRAIN. Avinoam Reches, Robert E. Burke, De-hua Jiang, H. Ryan Wagner, & Stanley Fahn, Dept. Neurology, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

Neurotensin (NT) injected intracerebroventricularly (ICV) in rat increases dopamine (DA) turnover in the corpus striatum and nucleus accumbens. Significant increases in 3,4-dihydroxyphenylacetic acid (DOPAC) levels occurred within 15 min after injection with peak levels at 60 min for both brain regions. This effect of NT was dose-dependent; significant increases in DOPAC and homovanilllic acid (HVA) accumulation were obtained at 3 ug with maximal response at 100 ug. NT, like haloperidol, stimulated 3,4-dihydroxyphenylalanine (DOPA) accumulation in striatal neurons in the presence of DOPA decarboxylase inhibitor after injection of gamma-butyrolactone (GBL), an agent which selectively blocks the firing of dopaminergic neurons. NT (30 ug) had a similar stimulatory effect on DOPA levels in the accumbens while haloperidol (0.25 $mg \cdot kg^{-1}$) had an insignificant effect in this brain region. NT failed to block the inhibitory effect of apomorphine (0.5 $mg \cdot kg^{-1}$) on DOPA accumulation in both the striatum and accumbens after GBL injection while haloperidol (0.25 $mg \cdot kg^{-1}$) inhibited apomorphine effect in both regions. Unlike antipsychotic drugs, NT failed to displace ^{3}H-spiperone from DA receptors in either the corpus striatum or nucleus accumbens. The presence of NT in the binding assay did not alter the ability of DA to displace ^{3}H-spiperone in either brain region. These experiments suggest that NT increases DA turnover in the nigrostriatal and mesolimbic pathways. It is suggested that this effect is mediated, in part, through inhibition of presynaptic DA receptors in a mechanism which is different from the one through which the antipsychotics interact with the DA receptors.

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160.4 VASOPRESSIN AFFECTS DOPAMINE-SENSITIVE ADENYLATE CYCLASE IN RAT BRAIN. N.D. Courtney* and M.A. Raskind* (SPON: T. Kennedy). Geriatric Research, Education & Clinical Center, Veterans Administration Medical Center, Seattle, WA 98108.

Evidence indicates that behavioral effects of vasopressin result from its action at specific sites (receptors) in the brain (De Wied, D. The Neurosciences. Edited by F.D. Schnidt et al, MIT Press, 1974, pp. 653-666). Peptide hormones may bind to receptors and consequently activate adenylate cyclase or may modulate the effect of other neurotransmitters on the activity of adenylate cyclase. We examined the effect of vasopressin on adenylate cyclase activity in rat brain, addressing the questions: 1) Does vasopressin alter adenylate cyclase activity in brain homogenates? 2) Does vasopressin modulate dopamine stimulation of caudate adenylate cyclase? Dopamine was chosen on the basis of its well-characterized stimulation of adenylate cyclase in homogenates of caudate nucleus.

Male Wistar rats were sacrificed by decapitation. The brains were quickly removed and brain regions were dissected according to the guidelines of Glowinski and Iverson. Brain regions were homogenized in 2 mM Tris-2 mM EDTA and assayed immediately. The standard adenylate cyclase assay mixture (100 µl) contained 80 mM Tris-Maleate pH 7.4, 0.5 mM ^{32}P ATP (150-250 cpm/pmol), 2.0 mM $MgCl_2$, 1 mM isobutyl methyl xanthine, 0.6 mM EGTA, 0.5 ml tissue homogenate plus the samples to be tested. The reaction was carried out at 30°C. ^{32}P cyclic AMP formed was recovered by the method of Salomon et al (Saloman, Y. et al. Anal. Biochem. 58:541-548, 1974).

Vasopressin significantly enhanced dopamine activation of caudate adenylate cyclase. This vasopressin effect was concentration dependent. Maximal enhancement (62%) occurred with $1x10^{-4}$ M vasopressin. Over the concentration range of $1x10^{-4}$ to $1x10^{-6}$ M, vasopressin alone had no reliable effect on the activity of caudate adenylate cyclase under the conditions tested. Further, adenylate cyclase activity was not reliably altered with vasopressin in selected brain regions.

These results indicate that although vasopressin may not have a direct effect on adenylate cyclase activity, it can modulate the activation of adenylate cyclase by another neurotransmitter. This modulatory effect is compatible with that demonstrated for other neuropeptides.

160.5 NIGROSTRIATAL DOPAMINE TERMINALS BEAR NEUROTENSIN RECEPTORS BUT THE MESOLIMBIC DO NOT. R. Quirion, H.D. Everist and A. Pert. Biological Psychiatry Branch and Neuroscience Branch, NIMH, Bethesda, MD 20205.

Neurotensin has been shown to exert complex effects on dopamine neurotransmission. These effects appear to be mediated through dopaminergic cell body areas as well as their terminal fields. The purpose of this study was to ascertain the precise localization of neurotensin receptors in relation to mesolimbic and nigrostriatal dopamine neurons. Nigrostriatal dopaminergic projections were lesioned unilaterally by intranigral injections of 6-hydroxydopamine (9 µg). Mesolimbic dopaminergic projections were lesioned either unilaterally (7.5 µg 6-OHDA) or bilaterally (7.5 µg 6-OHDA per side) by injections of this neurotoxin into the A-10 catecholamine nuclei. One month later tha animals were killed and their brains removed. Serial 25µm sections were taken through the forebrain region encompassing the nucleus accumbens and caudate nucleus and the midbrain region encompassing the substantia nigra and ventral tegmental area. These sections were than exposed to [^{3}H]-neurotensin and processed for autoradiography using a tritium sensitive film. Computer optical density analysis was used to quantify the lesion effects.

There appeared to be considerable binding of [^{3}H]-neurotensin to the substantia nigra, especially the zona compacta. Neurotensin receptors appeared to increase in number along a caudal to rostral plane in the caudate nucleus. No differences were observed along the dorsal-ventral plane however. The nucleus accumbens appeared to contain 24% more neurotensin binding than the head of the caudate nucleus at the same level. Neurotensin binding was reduced significantly in the substantia nigra following 6-OHDA lesions, indicating that some neurotensin receptors are located on dopaminergic cell bodies or dendrites. Similar findings have been reported by Palacios and Kuhar (Nature, 294: 587, 1981). Neurotensin binding also decreased in the more medial A-10 regions following 6-OHDA injections. We also found a substantial decrease of neurotensin receptors in the caudate nucleus ipislateral to the nigral 6-OHDA lesions. Neurotensin binding in the dorsal half of the caudate was reduced 65% relative to the unlesioned side. Binding in the ventral aspect, however, was reduced only 37%. Interestingly, neither unilateral nor bilateral A-10 lesions were effective in reducing neurotensin binding in the nucleus accumbens. Neurotensin receptors appear to be localized on dopaminergic terminals in the nigrostriatal but not the mesolimbic pathway. Dopaminergic cell bodies giving rise to both of these pathways, on the other hand, are high in neurotensin receptors. The pharmacological effects of neurotensin on dopaminergic neurotransmission are probably mediated through all of these sites.

160.6 EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE AND OTHER VASOACTIVE AGENTS ON ADENYLATE CYCLASE IN RAT CEREBRAL MICROVESSELS. Minta Huang* and O.P. Rorstad, Dept. of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

The following observations by other workers suggest that vasoactive intestinal polypeptide (VIP) may play a role in regulating cerebral vascular tone: 1. Vasodilatation of cerebral vessels and in increase of cerebral blood flow by VIP, 2. Localization of VIP-like immunoreactivity in nerves innervating cerebral vessels. On the basis that cAMP probably is the second messenger responsible for vasodilatation caused by beta adrenergic agents, the present study examined the effects of VIP and other vasoactive agents on the activity of adenylate cyclase (AC) in isolated rat cerebral microvessels. Catecholamines, prostaglandin E_1 (PGE_1), prostaglandin E_2 (PGE_2) and 2-chloro-adenosine (2-Cl-Ad) stimulated rat microvessel AC in a similar manner as previously observed with guinea-pig microvessel AC (Huang and Drummond, Mol Pharmacol 16: 462, 1979). The order of relative potency of the catecholamines was isoproterenol (INE)>epinephrine (EPI)>norepinephrine (NE) (see table). Stimulation of AC by catecholamines was completely abolished by propranolol and unaffected by phentolamine. Histamine and dopamine at 0.1 mM had marginal stimulatory action. Serotonin had no effect at the same concentration. Activations by PGE_1, PGE_2 and 2-Cl-Ad were dose-related. Only VIP, out of 22 peptides (many of which are vasoactive) examined, stimulated AC activity. Stimulation by VIP required guanosine triphosphate and was unaffected by propranolol or phentolamine. The magnitude of the VIP response was 2-3 fold over the activity observed with guanosine triphosphate alone. The activity of AC in response to VIP tested in combination with INE, PGE_1 or 2-Cl-Ad approximated the sum of activities elicited by each agonist tested independently. The action of VIP was not mimicked by other peptides of the glucagon-secretin family. Somatostatin-14, which has an inhibitory action on stimulated AC activity in some model systems, somatostatin-28 and [D-Trp^8,D-Cys^{14}] somatostatin (a superactive analog) had no effect on the stimulatory action of VIP or PGE_1 on microvessel AC. In conclusion, this study suggests the existence of cerebral vascular receptors for VIP that are distinct from receptors for catecholamines, PGE_1, adenosine or other peptides of the glucagon-secretin family.

Agent	ED_{50}(µM)
VIP	0.1
INE	0.45
EPI	1.8
NE	18
2 Cl Ad	19
PGE_1	>1
PGE_2	>10

(Supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research)

160.7 IMMUNOHISTOCHEMICAL LOCALIZATION OF GLUTAMATE DECARBOXYLASE AND MET-ENKEPHALIN-LIKE IMMUNOREACTIVITY IN THE SEPTAL COMPLEX OF THE RAT. D.L. Cheney, P. Panula*, A.V. Revuelta*, H.K. Thompson*, J.-Y. Wu and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032 and Baylor College of Medicine, Houston, Texas.

Previous biochemical and immunohistochemical studies have shown that met-enkephalin is found in the lateral septum, where enkephalin-immunoreactive fibers and terminals surround the cell bodies in a basket-like manner. However, few met-enkephalin immunoreactive cells have been found in this region and it has been suggested that the fibers might come from the hypothalamus or dorsal raphe nucleus. Antibodies against glutamate decarboxylase (GAD), the synthesizing enzyme of GABA, have been used to study the distribution of GABAergic neurons in different parts of the brain, but these studies have not included the septal region. In this study we report the distribution of met-enkephalin-like immunoreactivity (ME-LI) and GAD-like immunoreactivity in the septal complex of the rat. Brains of formalin-fixed normal and colchicine-treated rats were used. The peroxidase/antiperoxidase method was used to demonstrate immunoreactivity in fresh cryostat sections. Dilution of the specific antibodies was 1:1000 and incubation was carried out at 4° for 48 hours. Consecutive sections were incubated with non-immunized rabbit serum or met-enkephalin antiserum preabsorbed with met-enkephalin. These sections showed no reaction product. In normal rat brain, a dense network of met-enkephalin immunoreactive fibers and terminals surrounding the cells was seen in the intermediate part of the lateral septal nucleus. In colchicine injected animals, numerous met-enkephalin-immunoreactive cells were found in the dorsal, intermediate and ventral parts of the lateral septal nucleus. Fewer cells were found in the medial septal nucleus and in the nucleus of the diagonal band. Neurons exhibiting GAD-immunoreactivity were most numerous in the medial septal nucleus and in the nucleus of the diagonal band. These large cells extended posteriorly to the septo-fimbrial nucleus. The dorsal, intermediate and ventral portions of the lateral septal nucleus contained relatively few small GAD-positive cells, but in this area a dense network of GAD-positive fibers and terminals was observed. Thus, the GABAergic cell bodies in the septal complex are located in the same area where cholinergic septo-hippocampal neurons are found. Interactions between the GABAergic and cholinergic neurons in this area may explain the reduced turnover rate of acetylcholine in the hippocampus when GABA agonists are injected into the septal area. Contrary to what has been published it appears that the septal complex has a large number of met-enkephalin immunoreactive cell bodies and terminals. The possibility exists that enkephalin in the septum may be largely confined to septal interneurons.

161.1 TIME COURSE OF CAPSAICIN EFFECTS ON SUBSTANCE P-IMMUNOREACTIVE NERVES IN THE CARDIOVASCULAR SYSTEM. R.E. Papka, J.B. Furness* and M. Costa*. Dept. Anatomy, Univ. of Kentucky, Lexington, KY 40536 and Ctr. Neuroscience, Flinders Univ. South Australia 5042.

Capsaicin, the active agent in many chili peppers, releases and depletes substance P from primary sensory neurons. When administered to animals the effects of capsaicin are evident very quickly (within one to several minutes); these include lacrimation, activation of some nociceptors, bronchial constriction and vasodilatation. Since the effects of capsaicin are likely to result from release of substance P we examined, morphologically, the time course of the effects of capsaicin on substance P-immunoreactive nerves. Previous studies (Papka, R.E., et. al., Neurosci. Lett., 27: 47, 1981; Furness, J.B. et. al. Neurosci., 7: 447, 1982) indicated that guinea pig pericardium, atrioventricular valve, inferior vena cava, superior mesenteric artery and intestinal submucosa are model tissues for study because of their characteristic pattern and density of capsaicin-sensitive substance P-immunoreactive nerves. Guinea pigs were injected s.c. with capsaicin, 50 mg/kg, and sacrificed after 5,15,30, 60 minutes, 2,4,12 or 24 hours. Tissue was processed for immunohistochemistry of substance P (using whole mount preparations) and conventional electron microscopy.

In control preparations immunofluorescence in nerve trunks and fibers appeared bright and homogeneous. Within five minutes after injection of capsaicin changes were observable. The immunofluorescence began to appear less intense and less homogeneous (e.g. it appeared somewhat granular or "dusty"). Larger nerve trunks remained evident; however, there was a decrease in number of immunofluorescent fine single fibers. With increasing time after capsaicin treatment there was a trend toward progressive dimunition of the substance P-immunofluorescence, so that by 4 hours about 50% fewer nerve fibers were evident in the preparations and by 24 hours there was up to 95% fewer fibers. In those trunks that were evident, the intensity of immunofluorescence was markedly reduced and it appeared granular or "dusty". In animals treated for 2 hours or longer immunofluorescent swellings were commonly seen in nerve trunks. Electron microscopy revealed alterations in some nerve fibers within 5 minutes after capsaicin treatment. These include swelling of fibers, loss of internal morphology and disruption of mitochondrial morphology. In some fibers it appeared as though degenerative processes were in progress.

Capsaicin appears to have a quick onset of action with regard to its effects on the morphology of substance P-immunoreactive nerves. This action is progressive and by 24 hours depletion of substance P-immunoreactive material is nearly complete. Supported by Grant 1RO1HL22226 from NHLBI and PHS BRSG 2SO7RR05374-20.

161.2 ULTRASTRUCTURAL EFFECTS OF CAPSAICIN ON SUBSTANCE P TERMINALS IN THE SUBSTANTIA GELATINOSA. L.D. Wilkin, T.H. Williams. Dept. Anatomy, Univ. Iowa College Med., Iowa City, IA 52242.

Intrathecal or subcutaneous administration of capsaicin to experimental animals has been found to produce desensitization to noxious thermal and chemical stimuli. It has been hypothesized that capsaicin induced analgesia results from depletion of substance P (SP), a putative pain transmitter, from the terminals of primary afferent neurons in the spinal cord. In vivo and in vitro studies in which capsaicin was administered to spinal cord or spinal cord slices resulted in release and depletion of SP. However, other putative neurotransmitters have been identified in these areas and capsaicin has been shown to deplete somatostatin and cholecystokinin varicosities in the substantia gelatinosa. At the ultrastructural level, information is lacking regarding the effects of capsaicin on neurons containing SP.

Male guinea pigs (250-350g) received incremental subcutaneous injections of capsaicin totaling 125 mg over a 48 h period and were allowed to survive for 2 d or 8-10 d. Control animals received no treatment. All animals were anesthetized with Nembutal, ventilated with 95% O_2-5% CO_2, and perfused with a fixative solution of 4% paraformaldehyde-0.2% glutaraldehyde in phosphate buffer. Tissue blocks from the cervical spinal cord were sectioned with a vibratome and processed for SP immunocytochemistry according to the PAP method of Sternberger. Following immunostaining, tissues were postfixed in osmium tetroxide and embedded in epon for thin sectioning and electron microscopy.

At the light microscopic level, the normally dense SP immunoreactivity in layers I to III of the substantia gelantinosa appeared markedly reduced in both short- and long-term capsaicin treated animals. At the ultrastructural level, SP terminals were numerous in layers I to III of control animals, conforming to previous descriptions of small, unmyelinated fibers and C-type terminals. In 2d-survival animals, the contents of SP-immunoreactive axons and terminals in substantia gelatinosa were highly variable. While some of these profiles were intact, others appeared to be at various stages of disorganization with different degrees of depletion of SP-immunoreactive vesicles. In 8d animals, no intact SP-immunoreactive terminals were found; only disorganized SP-immunoreactive terminals devoid of vesicles were present. The axolemma was intact in most SP-immunoreactive elements. "Classical" degeneration of SP-immunoreactive processes was not seen.

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161.3 POTASSIUM EVOKED RELEASE OF CHOLECYSTOKININ FROM FRONTAL CORTEX AND HYPOTHALAMUS OF LEAN AND GENETICALLY OBESE RATS. P. Micevych, T. Yaksh*, V.L.W. Go* and J. Finkelstein. Depts. of Neurosurgery and Gastroenterology, Mayo Foundation, Rochester, MN 55905, and Program in Human Anatomy, Northeastern Ohio Univ. College of Med., Rootstown, OH 44272.

Recent evidence has implicated cholecystokinin (CCK) as a centrally acting satiety factor. We examined the tissue levels of CCK in the hypothalamus and frontal cortex of the genetically obese (fa/fa; wt = 450-500 g) Zucker rat and found that they were not different from age-matched lean (Fa/-; wt = 200-270 g) littermates. We then examined the release of CCK from frontal cortical and hypothalamic fragments in vitro. Rats of either sex, 9-14 weeks old were decapitated and the hypothalamus or frontal cortex was dissected out. Hypothalamic or cortical fragments were superfused with oxygenated KRB containing D-glucose and bacitracin (a protease inhibitor). The depolarizing stimulus was 50 mM K+. Five 10-min samples were collected, lyophilized and then reconstituted to 1/3 their original volume for RIA. The magnitude of release (basal or evoked) of CCK from frontal cortices obtained from fa/fa and Fa/- rats did not vary.

		Frontal Cortex	Hypothalamus
Tissue Levels[1]	Obese	26.7 ± 3.5	24.3 ± 5.7
	Lean	23.9 ± 4.1	27.6 ± 5.2
Release[2]	Obese	2.7 ± 4.1	2.1 ± 0.19
	Lean	2.6 ± 3.5	1.15 ± 0.16

[1]Values are mean of 5 trials ± SEM in pMole equivalents CCK-8/mg wet weight tissues.

[2]Mean of 9 trials ± SEM in fMole equivalents CCK-8/mg/10 min.

However, hypothalamic fragments from obese rats were observed to release significantly greater amounts of CCK, in response to K+, than their age-matched lean littermates. The basal release levels from obese and lean rats were the same (0.3 ± 0.09 fMoles/mg tissue/10 min). Sephadex G-50 chromatography demonstrated that the immunoreactive CCK was isographic with authentic sulfated CCK-8. These studies, and the recent work demonstrating that the levels of CCK-8 binding sites are lower in the hypothalami of obese rats than in lean rats (Finkelstein et al, Endocrine Soc. Abs., 1982) may imply a genetic lesion in these obese animals which involves a decreased receptor population in hypothalamus and an elevated release of CCK in the hypothalamus, a CNS area implicated in satiety. (Supported by AM 07198-06, NS 16541 and Mayo Foundation.)

161.4 IMMUNOHISTOCHEMICAL AND RADIOIMMUNOASSAY STUDIES OF SUBSTANCE P IN THE DORSAL HORN FOLLOWING GANGLIONECTOMY. A. Stroink*, P. Micevych, V.L.W. Go* and T.L. Yaksh* (SPON: F.W.L. Kerr). Depts. of Neurosurgical Research and Gastroenterology, Mayo Foundation, Rochester, MN 55905.

Deafferentation of the cat lumbosacral dorsal horn by unilateral ganglionectomy L2-S3 produces a depletion of substance P (sP) immunoreactivity as demonstrated by the peroxidase-antiperoxidase technique of Sternberger. This depletion is most marked at 11 days postlesion as compared to the nonlesioned side. This was followed by a partial recovery of sP in the substantia gelatinosa at 14 days; and by one month the restoration of sP in the dorsal horn was indistinguishable from the unlesioned side. Met-enkephalin (M-ENK) immunoreactivity remained unchanged by ganglionectomy. We have attempted to correlate these qualitative immunohistochemical results, thought to be suggestive of sprouting, with quantitative radioimmunoassay (RIA) analysis. Unilateral ganglionectomies L2-S3 were performed on cats (2.5-4 kg) which were allowed to survive 7, 11 and 21 days postsurgery and then sacrificed under deep pentobarbital anesthesia. Spinal cord tissue was then removed at the midpoint of the surgical lesion (L4-L7) and separated into four quadrants (left and right, ventral and dorsal) and immediately frozen. RIA was performed on this tissue as well as comparable spinal cord tissue in unlesioned cats. The control levels of sP and M-ENK in the dorsal horns of unoperated cats (ng/gm) are given below:

	Right Dorsal Horn	Left Dorsal Horn
sP	197.7	187.2
M-ENK	44.7	41.3

Compared to the intact dorsal horn, a 35% depletion of sP immunoreactivity is observed at 7 days postlesion, 29% depletion of sP at 11 days, and 70% depletion of sP at 21 days. At no time following unilateral ganglionectomy were levels of M-ENK immunoreactivity altered as compared to the controls. There was no significant change in the levels of sP immunoreactivity in the ventral horns in any of these animals. RIA does not appear to confirm the immunohistochemical evidence for the recovery of sP immunoreactivity following ganglionectomy. The reasons for this discrepancy are yet unclear but may question our understanding of sprouting in the dorsal horn postdeafferentation. (Supported by funds from the Mayo Foundation and AM 07198-06.)

161.5 DISTRIBUTION OF ENKEPHALIN, SUBSTANCE P AND SEROTONIN LIKE IMMUNOREACTIVITY IN THE INTERPEDUNCULAR NUCLEUS OF THE CAT. S.E. Kapadia* and N.C. de Lanerolle. Depts. of Anatomy and Neurosurgery, Yale Univ. Sch. of Medicine, New Haven, CT 06510.

The aim of this investigation was to define the distribution of methionine-enkephalin (ENK), substance P (SP) and serotonin (5-HT) like immunoreactivity within the interpeduncular nucleus (IPN). Consecutive vibratome transverse serial sections (65 um) obtained in the cranio-caudal axis of the midbrain were processed using the indirect antibody peroxidase-antiperoxidase immunocytochemical technique, and examined by both light and electron microscopy (EM). The IPN can be subdivided by the cross sectional arrangement of blood vessels within it. Two rows of dorsoventrally aligned large blood vessels are located bilaterally and two similarly aligned rows of smaller vessels are present medial to the large vessels, dividing the IPN into a paired outer lateral region, a paired intermediate region and an unpaired median region. Each region is further divided into a dorsal, middle and ventral zone. ENK-like immunoreactivity was seen in some cell bodies and fibers within the paired lateral and intermediate regions throughout the length of the IPN. About the middle of the IPN in the cranio-caudal axis was a distinct cluster of ENK cell bodies and fibers in the dorso-intermediate zone. SP containing perikarya and fibers were seen mainly in the rostral IPN with maximal concentrations in the unpaired median region, specifically in the ventromedian and central zones. By contrast, 5-HT labeled structures were principally located in the caudal IPN. Both cell bodies and fibers were distributed throughout the lateral and intermediate regions extending into the dorsomedian and ventromedian zones. Thus the 5-HT immunoreactive structures form a girdle inside the IPN around its periphery. In the dorsomedian zone were predominantly 5-HT cell bodies, whereas in other regions a mixture of cell bodies and fiber-like processes were seen. EM revealed distinct differences in cell size, morphology of cellular organells and synaptic organization of the different neurochemical systems examined. Of particular interest was the clear demonstration of axo-dendritic contacts in which both the terminal and dendrite were immunoreactive for SP. (Supported by Yale Fluid Research Funds).

161.6 CONTRIBUTION OF BULBOSPINAL AND INTRASPINAL PATHWAYS TO SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE THORACIC SPINAL CORD. J.F. McKelvy B.M. Davis, J.E. Krause and J.B. Cabot, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, N.Y. 11794.

Substance P-like immunoreactivity (SPLI) is found throughout the thoracic spinal grey, and is particularly dense in laminae I and II of the dorsal horn and in the neuropil surrounding sympathetic preganglionic neurons (SPN's). Since both the brainstem and the spinal cord contain SP positive cells which might give rise to such terminations, the relative contribution of bulbospinal and spinal-spinal pathways to SP content in the thoracic spinal cord was assessed using a highly specific and sensitive radioimmunoassay.

White Carneaux pigeons (15) were used. In 6 birds the thoracic spinal cord was totally transected at the junction between T2 and T3. After a 7 day survival time the birds were decapitated. T1, T2, T3 and T4 were removed, frozen separately on dry ice and microdissected into three regions: dorsal horn (DH), ventral horn (VH) and the intermediate spinal grey (containing the SPN's). Unoperated control birds (6) were processed in parallel. Three additional birds received mid-cervical hemisections. After 14 days these birds were decapitated, and the T1 spinal segment was removed and frozen. T1 was microdissected in the transverse plane into right and left DH and VH, and right and left intermediate laminae. SP was acid extracted (cold 2M HOAc) from tissue and acid soluble protein in each sample was determined using Lowry's assay. The results are expressed in SP/region (e.g.SP/T1 DH) and SP/ug acid soluble protein.

In general results were similar whether the amount of SP was expressed per region or per ug protein. Significant depletion was seen in the DH and VH in all segments sampled, rostral and caudal to the thoracic transection. Depletion in the DH was 30-50% (ANOVA, $p<.05$) and 30-40% in the VH ($p<.05$). No significant depletion was seen in intermediate laminae rostral to the transection. Caudal to the transection, however, the amount of SP was decreased by as much as 37% ($p<.05$). No significant differences in SP content could be detected between the two halves of the spinal grey in cervical hemisected birds. Indeed, the total amount of SP calculated by combining samples from both sides of the spinal cord, was indistinguishable from levels found in controls.

The lack of significant SP depletion following cervical hemisection suggests that bulbospinal SP input to avian DH, VH, and SPN's must be minor. In contrast, the presence of significant SP inputs via intraspinal circuits can be inferred from the observations following total transection of mid-thoracic spinal cord. (Supported by HL24103 and BNS 7921781.)

161.7 SUBSTANCE P PATHWAYS TO SYMPATHETIC PREGANGLIONIC NEURONS: EVIDENCE FOR MAJOR SPINAL-SPINAL CIRCUITRY. B.M. Davis and J.B. Cabot, Dept. Neurobiology and Behavior, S.U.N.Y. at Stony Brook, Stony Brook, NY 11794.

Substance P (SP) immunoreactivity has been localized to the sympathetic preganglionic neuropil at the light microscopic level in several vertebrates including the cat, rat, and pigeon. Moreover EM immunohistochemistry has revealed SP staining in terminals containing dense core vesicles in the rat sympathetic preganglionic neuropil (Holet and Elde,1981). We report here the results of experiments designed to locate the cells of origin of the SP projection(s) to avian sympathetic preganglionic neurons (SPN's).

White Carneaux pigeons (Columba livia) were used. Lesions were placed so as to isolate the thoracic spinal cord from regions of the nervous system known to contain SP cells. After a 7 or 14 day survival time, birds were perfused and spinal cord segments sectioned and stained for SP or 5-HT-like immunoreactivity. In an additional 3 animals injections of tritiated amino acids were made in dorsal root ganglia and the appropriate segments of thoracic spinal cord were processed for autoradiography.

Pigeons in which 3,5 or 6 sequential dorsal rhizotomies (C14-T5) were performed showed no obvious depletion of SP staining in the SPN neuropil. Conversely, SP staining was markedly reduced in the dorsal horns ipsilateral to the rhizotomies. The autoradiographic results confirmed that primary sensory afferents terminated in dorsal and ventral horns but no projection to SPN's could be detected.

High cervical hemisections also had no effect on the SP staining. In these cases, adjacent sections were stained for 5-HT-like immunoreactivity.(The cells giving rise to 5-HT terminals in the spinal cord are located exclusively in the brainstem.) Unlike the SP staining, 5-HT staining was greatly attenuated in the dorsal and ventral horns, and in the SPN neuropil ipsilateral to the hemisection.

Minor SP depletion was observed in all regions of the spinal grey, rostral and caudal to a mid-thoracic spinal transection. Depletion was confined to the segments adjacent to the transection, with normal levels of SP staining present in the remainder of the spinal cord. Substantial depletion of SP within the SPN neuropil was observed only when thoracic spinal cord was totally isolated from ascending and descending axons by two ipsilateral thoracic hemisections or two total thoracic transections.

Currently, we are repeating these experiments using a highly specific and sensitive radioimmunoassay for SP(1-2pg).The results to date confirm the major conclusion of the above experiments: The majority of SP terminations within the SPN neuropil have their cells of origin in the spinal grey.(Supported by HL24103)

161.8 REGIONAL BRAIN DISTRIBUTION OF NEUROTENSIN-LIKE IMMUNOREACTIVITY IN THE WOODCHUCK (MARMOTA MONAX). G. Bissette*, D. Luttinger, C.B. Nemeroff, V.M. Miller and A.J. Prange, Jr. (SPON: P.T. Loosen). Biol. Sci. Res. Ctr., Dept. Psychiat. and the Neurobiology Program, University of North Carolina School of Medicine, Chapel Hill, NC 27514 and School of Life and Health Sciences, University of Delaware, Newark, DE 19711.

Neurotensin (NT) is a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Lle-Leu-OH) that is heterogeneously distributed in the central nervous system (CNS) of the rat, guinea pig, rabbit, cow, monkey and man. When administered directly into the CNS, NT produces a variety of effects including hypothermia, analgesia and muscle relaxation (see Trends in Neurosciences 3: 212-215, 1980 for review). Our group has previously reported that centrally administered NT produces a potent hypothermic response in the rat, mouse, gerbil, hamster, guinea pig and monkey (Pharmacol. Biochem. Behav. 11: 473-477, 1979). This effect was not observed in the woodchuck, even after the administration of large doses (100 µg) of the peptide. In view of this finding, we sought to determine whether NT is present in the brain of the woodchuck and if so, whether the CNS distribution of the peptide is similar to that observed in other mammals. Adult woodchucks (3 male, 5 female) were killed and their brains rapidly removed, dissected and frozen on dry ice. The samples were homogenized in approximately 10 volumes of 1N HCl and NT was measured by radioimmunoassay (Manberg et al., J. Neurochem., in press) using an antiserum directed towards the mid-portion of the NT molecule. The sensitivity of the assay was 20 pg per tube. Protein concentration was determined with the Folin reagent and the data obtained expressed as pg NT per mg protein. The highest concentration of NT was found in the hypothalamus. The following brain regions contained substantial quantities of immunoreactive NT (expressed in decreasing concentrations): septum, amygdala, caudate-putamen, ventral tegmentum, cingulate cortex, and olfactory tubercle. The pituitary contained no detectable NT. The CNS distribution of NT in the woodchuck is similar to that observed in the rat, monkey and man.

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161.9 PREFERENTIAL IMMUNOHISTOCHEMICAL LOCALIZATION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN THE SACRAL SPINAL CORD OF THE CAT. C. N. Honda. Neurobiology Curriculum and Department of Physiology, Univ. of North Carolina, Chapel Hill, NC 27514.

The distribution of neuronal processes containing VIP-like immunoreactivity was compared with that of substance P (SP)-, somatostatin (SS)-, and cholecystokinin-8 (CCK)-like immunoreactivities in vibratome sections of lumbar, sacral, and coccygeal segments (L4-Co4) of the cat spinal cord. The peroxidase-anti-peroxidase (PAP) technique was used after permeabilization with ethanol.

VIP-containing fibers were visualized in some sacral dorsal rootlets, their spinal entry zones, and often in the dorsolateral funiculus. Lissauer's tract (LT) was intensely labelled in sacral segments, but the proportion of LT occupied by VIP fibers progressively decreased rostrally and caudally until only small bundles persisted at L4 and Co4. Forming a thin shell around the dorsal horn, collaterals, apparently originating from LT, projected either medially or laterally through lamina I. Laterally, many axons terminated in lamina V, and some in lateral laminae VI and VII. Others projected further to medial V and the posterior commissure. Medially, VIP axons descended through lamina I along the lateral edge of the dorsal funiculus to expand into terminal fields in the dorsal commissure and medial lamina V. VIP fibers and terminals were also present in the gray matter surrounding the central canal. The spinal distribution of VIP fibers and terminals was most dense in the sacral segments.

This distribution of VIP-containing fibers is remarkably similar to that of pelvic nerve visceral afferents (Morgan et al. J. Comp. Neurol. 201:415,1981), both in their segmental distribution patterns within the gray matter, and in their localization to the sacral cord. SP-, SS-, and CCK-containing fibers and terminals exhibited segmental localizations within the gray matter similar to pelvic nerve afferents and VIP fibers, with the exception that SS was well localized to the inner portions of lamina II and the outer parts of III. The density of SP-, SS-, and CCK-containing fibers was constant at all levels examined (L4-Co4). In contrast, the distribution of VIP-containing fibers, much like the pelvic nerve afferents, was mostly confined to the sacral segments.

Thus, while SP-, SS-, and CCK-containing fibers may contribute to the population of sacral visceral afferents, they appear also to be common to afferent systems in other segments. It is suggested that VIP-containing fibers are preferentially associated with the spinal distribution of an afferent system, such as the pelvic visceral nerves, which is localized to the sacral spinal cord.

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161.10 VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN PRIMARY AFFERENT PROJECTIONS TO THE SACRAL SPINAL CORD OF THE CAT. M.Kawatani*, I. Lowe, C.Morgan, I.Nadelhaft, S.Erdman* and W.C.deGroat. Depts. of Pharmacol. and Neurosurg., Univ. Pitt. and V.A. Hosp., Pitt., PA

HRP studies have shown that sacral afferent neurons innervating the urogenital organs and colon via the pelvic nerve send axons centrally into Lissauer's Tract (LT) and collaterals into Lamina I laterally and medially around the dorsal horn (DH). The lateral collateral pathway (LCP) projects into the sacral parasympathetic nucleus (SPN). In the present study we have used immunohistochemical (IHC) techniques to examine the relationship of VIP to these visceral afferent pathways since VIP has been proposed as a transmitter in primary afferent neurons and in addition is very abundant in nerve fibers in the pelvic organs.

The distribution of VIP in lumbosacral and coccygeal segments was determined with IHC techniques Tissue was obtained from normal adult cats as well as cats in which sacral ventral and/or dorsal roots were transected on one side 4-5 weeks prior to sacrifice. In sacral segments VIP terminals were concentrated in LT and lamina I in the area of the LCP. In longitudinal sections VIP in this region exhibited a periodic organization in which clusters of terminals occurred at fairly regular intervals (210-250 μ) along the length of the cord. Pelvic visceral afferents had a similar periodicity. A few VIP axons projected from lateral lamina I through the dorsal band of the SPN in lamina V to end in the region of the dorsal commissure and central canal. Few VIP fibers were noted in the lateral band of the SPN. Small numbers of VIP axons projected medially from LT into lamina I and along the medial side of the DH into the dorsal commissure. VIP axons were noted in the region of the central canal in sacral segments. The L7 and Cxl segments exhibited fewer VIP axons in LT, lamina 1 & X, whereas other lumbar and coccygeal segments were virtually devoid of VIP. VIP fibers were markedly reduced in LT and laminae I & V ipsilateral to where sacral dorsal and ventral roots were transected. A slight reduction in VIP fibers was also noted following transection of the ventral roots. VIP around the central canal was not changed following deafferentation.

These experiments have shown that in lumbosacral and coccygeal segments of the cat spinal cord VIP is contained largely in afferents entering the cord at the sacral level. Since the distribution of VIP axons in LT, lamina I & V closely parallel the distribution of previously demonstrated visceral primary afferent projections, it is tempting to speculate that VIP is contained in part in visceral afferents. Further support for this proposal has been obtained in preliminary studies in which dye tracing was combined with IHC. In these experiments it was shown that some sacral dorsal root ganglion cells labelled by true blue applied to the pelvic nerve also contained VIP.

161.11 AN INVESTIGATION OF NEURONS DISPLAYING CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY IN THE CEREBRAL CORTEX OF THE MONKEY AND RAT. S.H.C. Hendry, K.L. Valentino, E.G. Jones and M.C. Beinfeld. James L. O'Leary Division of Experimental Neurology and Neurological Surgery and McDonnell Center for The Study of Higher Brain Function, Washington University School of Medicine, and Department of Pharmacology, St. Louis University, St. Louis, MO

Light and electron microscopic immunocytochemistry was used to examine the neurons of the monkey and rat cerebral cortex that display cholecystokinin-like (CCK) immunoreactivity. In both species the CCK positive cells form a very small percentage of the total population of cortical neurons. The somata of the immunocytochemically stained neurons are present in all layers but are concentrated superficially, in layers I-III. CCK positive neurons are all non-pyramidal neurons with somata among the smallest found in neocortex. Most give rise to 2-4 long, radially ascending and descending processes, which branch at relatively few points and can extend through much of the thickness of the cortex. Labeled punctate structures that possibly correspond to CCK positive axon terminals are located in several layers and are most densely distributed in layer VI.

Ultrastructurally, CCK positive neurons resemble typical non-pyramidal cells of previous descriptive studies. They receive both symmetric and asymmetric synapses on their somata and dendrites. The numbers of axosomatic and axodendritic synapses on different CCK positive neurons are found to vary. CCK positive terminals form asymmetric synapses onto several different neuronal processes including the somata of unlabeled non-pyramidal cells and the shafts of dendrites. Within CCK positive terminals, reaction product is present diffusely in the cytoplasm and could not be localized to any distinct population of synaptic vesicles.

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161.12 CHOLECYSTOKININ-OCTAPEPTIDE-IMMUNOREACTIVITY (CCK-8-LI) IN NEURONS IN DISSOCIATED CULTURES OF RAT CEREBRAL CORTEX. John Delfs, Eugene Straus, Zhu Chun-Han*, and Marc Dichter. Division of Neuroscience, Children's Hosp.Med. Center, Boston, MA 02215 and Dept. of Medicine, Montifiore Hosp., Bronx, N.Y.

The carboxy-terminal octapeptide of cholecystokinin (CCK-8) has been demonstrated by radioimmunoassay to exist in high concentrations in mammalian cerebral cortex (Muller et al., 1977; Rehfeld, 1978) and immunohistochemical studies in vivo suggest a localization to a neuronal population (Straus et al., 1977; Larsson and Rehfeld, 1979).

Dissociated cultures of cerebral cortex were prepared from 15-16 day fetal rats as previously described (Dichter, 1978; Delfs et al., 1980). A well-characterized radioimmunoassay was used to determine the presence of CCK-8-LI at time intervals from plating to 35 days in culture. Both the cells and the media contained insignificant amounts of CCK-8-LI at plating, but demonstrated a time-dependent increase in the amount of CCK-8-LI.

For immunohistochemical staining, cultures grown on glass cover slips were fixed in 4% paraformaldehyde followed by 0.4% Triton-X-100. After preincubation with non-immune goat serum, the culture was incubated with a rabbit antibody prepared against a CCK-8 conjugate (Immunonuclear Corp.) at concentrations from 1:100 to 1:400, followed by a goat anti-rabbit gammaglobulin (Cappell) and were studied under a Zeiss combination phase-fluorescence microscope. Staining was considered specific when eliminated by preincubation with an excess of synthetic CCK-8 (sulfated, Peninsula Labs).

Neurons containing CCK-8-LI were predominately of two categories. The most prominent staining was seen in widely ramifying processes, with brightly staining swellings in close anatomical relation to other neuronal perikarya and dendrites. These processes may arise from large, multipolar neurons, better seen after pretreatment of the cultures with colchicine 100uM for 46 hours prior to fixation. Staining for CCK-8-LI was also seen in very small neurons of both stellate and bipolar morphology, which were often in intimate anatomical proximity to much larger non-CCK neurons.

These results indicate that CCK-8 is synthesized by several different morphologic subtypes neurons in these cultures and this system may be valuable for further studies on the synthesis and role of CCK-8 in cerebral cortex.

161.13 THE DEVELOPMENT AND DISTRIBUTION OF CHOLECYSTOKININ AND AVIAN PANCREATIC POLYPEPTIDE IMMUNOREACTIVE NEURONS IN THE RAT VISUAL CORTEX. J.K. McDonald, J.G. Parnavelas, and A.N. Karamanlidis. Depts. of Physiology and Cell Biology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

Using immunocytochemistry, we have examined the development of cholecystokinin-like (CCK-IMR) and avian pancreatic polypeptide-like (APP-IMR) immunoreactivity in the rat visual cortex. Sprague-Dawley albino rats of both sexes at several postnatal ages, as well as adults, were perfused with fixative containing 4% paraformaldehyde, 0.1M D,L-lysine and 0.01M Na periodate in 0.1M phosphate buffer. 35 µm coronal sections were cut and processed for fluorescence and PAP immunocytochemistry. CCK and APP antisera were generously provided by G. Rosenquist and J. Kimmel. Preabsorption of each antiserum with the appropriate antigen prevented labeling.

In the adult visual cortex, CCK-IMR neurons were located in all cortical layers but were concentrated primarily in layers II & III. Labeled multipolar and bitufted nonpyramidal neurons were observed, the majority resembling bitufted neurons described in Golgi preparations (Feldman & Peters, 1978). Of particular interest was the presence of labeled neurons in layer I which correspond to the persisting horizonal cells and vertical cells described by Bradford et al. (1977). Immature CCK-IMR cells were first observed on the third postnatal day. They matured gradually and increased in frequency during the next few days and by day 8, bitufted and multipolar neurons were identified in all cortical layers, particularly layers II & III. Labeled cells and their processes mature dramatically during the second postnatal week and only slight changes were noted after this time.

APP-IMR neurons in the adult were fairly evenly distributed in layers II to VI but appeared more frequently in layers V & VI. Labeled cells included multipolar, bitufted and bipolar forms of non-pyramidal neurons. A very small number of pyramidal cells were also identified in layers V & VI. An interesting feature of some bitufted neurons and layer VI multipolar neurons with horizontally oriented dendrites was their resemblance to somatostatin IMR cells reported in the rat visual cortex (McDonald et al., 1982). A small number of immature APP-IMR neurons were present in the subplate region on the first postnatal day. These cells mature gradually during the first two weeks of postnatal life. At the end of this time, APP-IMR neurons appeared similar in morphology to their adult counterparts. These results indicate that various peptide-containing immunoreactive neurons in the rat visual cortex display distinctive morphologies, distributions and rates of development. (Supported by NIH Grants EY02964 and EY03783).

161.14 LOCALIZATION AND CHARACTERIZATION OF GLUCAGON-LIKE IMMUNOREACTIVITY IN THE RETINA. N. Brecha, M. Cilluffo* and T. Yamada*. Center for Ulcer Research and Education, VA Wadsworth Medical Center, Jules Stein Eye Institute, Brain Research Institute and UCLA School of Medicine, Los Angeles, CA 90073.

Previous studies localizing glucagon-like immunoreactivity in the central nervous system including the retina have suggested that glucagon-like immunoreactivity is of the non-pancreatic type. We have extended these studies in retina to further characterize the morphology and biochemistry of retinal glucagon-like immunoreactivity. Pigeon retinas were fixed in paraformaldehyde/lysine/periodate solution, washed, and processed according to standard immunohistochemical protocols. Using antisera raised against porcine pancreatic glucagon (N211 provided by N Track, and G-VI) at least 3 distinct populations of amacrine cells were identified. In central retina the majority of immunoreactive cells had a small somatic diameter and gave rise to primary processes which arborized in lamina 1 of the inner plexiform layer (IPL). Some of these processes contributed secondary processes which arborized in lamina 3 and perhaps lamina 5 of the IPL. A second population of medium- to large-sized immunoreactive cells located in ventral retinal regions gave rise to single thick primary processes which coursed in lamina 1 of the IPL for a short distance before ramifying into many fine secondary processes. These processes ramified in laminae 1, 3 and 5 of the IPL. One of these processes, present in lamina 1 of the IPL was followed to the ora serrata where it joined a fascicle of glucagon-containing fibers. A third population of immunoreactive cells was present in the peripheral retina. These cells had medium-sized somata and gave rise to two or three primary processes which ramified primarily within lamina 1 of the IPL, some of these processes also extended to the ora serrata. No staining was observed in sections incubated with antisera preabsorbed with 10 µM synthetic glucagon. Acid-ethanol extracts of retinal tissues were radioimmunoassayed using antiserum 30K which is specific for the COOH-terminus of glucagon. Retinal GLI was 10.0±1.2 pmol/g wet weight (mean±SE) as compared to 2737±185 pmol/g in the pancreas and undetectable quantities in the brain. Retinal GLI chromatographed on Sephadex G-50 as a smaller molecule (K_d 0.85) than did pancreatic GLI (K_d 0.70) which co-eluted with porcine pancreatic glucagon.

Our studies demonstrate that 1) GLI is localized in pigeon retina to at least 3 distinct types of amacrine cells, and 2) retinal GLI is of the pancreatic type although it may be somewhat smaller in size.

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161.15 COEXISTENCE AND RELEASE OF PANCREATIC POLYPEPTIDE FROM NORADRENERGIC SUPERIOR CERVICAL GANGLIA NEURONS. J. A. Olschowka and D. M. Jacobowitz. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

Pancreatic polypeptide (PP) is a 36 amino acid peptide recently found in neuronal cells in the periphery and the brain. Its neurophysiological signficance is unknown. However, PP-like immunoreactivity has been demonstrated to coexist with catecholamine-containing cells of the rat hindbrain and periphery. This study examines the coexistence of PP in noradrenergic (NA) neurons of the rat superior cervical ganglia (SCG) and the release of PP from these neurons.

Using the indirect immunofluroescent technique, bovine PP-like immunoreactive fibers and cell bodies were observed in the SCG only after increasing the intraganglionic content of PP by either: 1. direct application of colchicine on the SCG (75 µg in 25 µl saline), 2. vinblastine treatment (3 mg/kg, iv) or 3. ligating the pre- or postganglionic nerves. The SCG was observed to contain a dense intraganglionic plexus of PP-like immunoreactive fibers and 30-50% of all SCG neurons contained PP. Thick PP-containing fiber bundles were observed at both the pre- and postganglionic poles of the SCG. Moderate numbers of PP-fibers were observed both entering and leaving the SCG through the preganglionic nerve, while large numbers of fibers left with the postganglionic nerves. These postganglionic PP-fibers could be observed innervating the vasculature of the eye and salivary gland and disappeared after lesion of the nerve. 6-OHDA treatment (2 x 50 mg/kg, ip), which selectively destroys NA fibers, also resulted in a loss of these fibers.

Prior studies have shown that NA is released from varicose terminals during high nerve impulse activity. To determine if PP is co-released with NA during stimulation, the right cervical sympathetic trunk was separated from the vagus, ligated, severed and supramaximally stimulated for 20 or 40 min (5 V, 20 pps, 10 msec duration). The left sympathetic trunk was severed as control. A few animals were pretreated with reserpine (5 mg/kg, iv) 5 hr prior to stimulation. Immediately after stimulation, the animals were perfused and processed for immunohistochemistry. On the stimulated side, the PP-fibers of the eye and salivary gland were virtually depleted of their PP content as compared to control. The presence of reserpine, while totally depleting NA, did not influence the control side or enhance the reduction of PP on the stimulated side. These results suggest that PP coexists with a proportion of SCG catecholamine cells and that it is co-released with NA during stimulation.

161.16 IMMUNOREACTIVE ANGIOTENSIN II IN THE BRAIN OF THE DOCA-SALT HYPERTENSIVE RAT. J.A. Weyhenmeyer. College of Medicine and Department of Anatomical Sciences, University of Illinois, Urbana, IL 61801.

Angiotensin II (AII) has been proposed to play an important role in the neural control of blood pressure, drinking behavior, naturesis, and hormone release. Although the origin of brain AII has remained controversial, this laboratory and others have demonstrated the presence of immunoreactive AII in cell bodies and fiber profiles in the rat and primate CNS. Recently, we reported that there was a significant increase in the distribution of AII in the brain of the spontaneously hypertensive rat (SHR) compared to its normotensive control, the Wistar Kyoto rat (Hypertension, 1982). The purpose of this investigation was to determine whether the distribution of AII in the brain and brainstem of the DOCA-salt hypertensive rat was similar to that observed in the genetically predisposed SHR.

Ten-week-old male Sprague Dawley rats were given weekly injections of DOCA (30 mg Percorten pivalate/kg body weight) and their drinking water was substituted with a 1% NaCl solution. Rats were monitored for blood pressure by non-invasive techniques, and only those animals with a systolic pressure above 160 mmHg were considered acceptable for experimental studies. AII was localized by the unlabeled antibody enzyme method of Sternberger. Briefly, rat brains were fixed in buffered picric acid-paraformaldehyde, vibratome sectioned, and incubated in Triton x-100 to enhance antibody penetration. The sections were incubated with rabbit anti-AII, followed by goat anti-rabbit IgG and rabbit peroxidase anti-peroxidase. Staining was completely eliminated by the substitution of primary antiserum preabsorbed with excess AII or when preimmune serum was substituted for the primary antiserum.

Immunoreactive AII was widely distributed in fiber profiles of the brain and brainstem of the DOCA-salt hypertensive rat. Many of these nerve fibers contained densely stained varicosities along the length of the profiles. Dense concentrations of AII containing fibers were localized in structures immediately surrounding the lateral, third and fourth ventricles, which is consistent with observations in the SHR. Positively stained fiber profiles were also observed in structures that have been implicated in the neural control of blood pressure, including the locus coeruleus and nucleus tractus soitarius. In contrast to the AII fiber pattern in the brains of the SHR, relatively few fibers containing AII have been localized in the preoptic area or thalamus of the DOCA-salt hypertensive rat.

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161.17 ANGIOTENSIN II-LIKE IMMUNOREACTIVITY IN THE CENTRAL NUCLEUS OF THE RAT AMYGDALA. M.D. Cassell, T.S. Gray, D. Ganten and T.H. Williams. Univ. of Iowa, Iowa City, IA 52242.

The immunohistochemical localization of angiotensin II-like immunoreactivity (AII) in the CNS and its demonstrated physiological effects on neurons suggest that angiotensin II could act as a neurotransmitter. The central nucleus of the amygdala (CNA) contains several other neuropeptides, each with a highly specific pattern of distribution of cell bodies and terminals. AII has been identified in the amygdala and, with a view to analyzing the relationships to other peptide systems, we have determined the precise distribution of AII in the CNA at both the LM and EM level using a modified Sternberger-PAP immunocytochemical method. Anti-AII was generated in rabbits. Immunoadsorption of the antibody with synthetic val^5 AII, but not angiotensin I, lys-vasopressin or neurotensin, abolished immunostaining.

At the mid-rostrocaudal level, a large number of AII terminals were distributed over the lateral and intermediate parts of the CNA. This distribution is quite distinct from the distributions of substance P, VIP, somatostatin, CCK, neurotensin and m-enkephalin immunoreactive terminals. AII-terminals outlined cell bodies and dendrites, particularly in the intermediate CNA. These rings and clusters of terminals made definitive identification of AII cells in the CNA very difficult. The high density of AII terminals was continued into the rostral CNA but was distinct from the anterior amygdaloid area which had few AII terminals. AII-fibers were seen traveling in the adjacent substantia innominata but lesioning of this area produced only a slight reduction in AII terminal density in the CNA. Caudally, AII terminals were present throughout the CNA and fibers could be seen passing into the stria terminalis. A small number of AII terminals were observed in the medial, cortical, basomedial and basolateral nuclei, but not in the lateral nucleus.

Ultrastructurally, AII was observed in terminals, dendrites and cell bodies. In perikarya and dendrites, the reaction product was distributed in the cytosol against mitochondria and rough ER, and in a few large vesicles. In terminals, reaction product was associated with the surface of round, clear vesicles. Large, dense cored vesicles were also observed. AII terminals were observed making asymmetrical axo-dentritic and axosomatic synaptic contacts with non-AII neurons. AII-terminals were observed making symmetrical axosomatic, but not axodendritic, contacts with AII-perikarya.

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161.18 EGG-LAYING HORMONE, LEUCINE-ENKEPHALIN AND SEROTONIN IMMUNOREACTIVITY IN THE ABDOMINAL GANGLION OF APLYSIA: A LIGHT MICROSCOPIC STUDY. W.E. Hopkins*#†, L.S. Stone*#, B.S. Rothman#, A.I. Basbaum#§, and E. Mayeri#† (SPON: E. Glazer). Depts. of #Physiology and §Anatomy, Univ. of Calif., San Francisco, CA 94143 and †Dept. of Basic Sciences, Calif. Coll. Podiatric Medicine, San Francisco, CA 94115.

Immunoreactive egg-laying hormone (IR-ELH), leucine-enkephalin (IR-ENK), and serotonin (IR-5HT) cells and processes were studied in the abdominal ganglion of the marine mollusk Aplysia californica with emphasis on the anatomical relationships of immunoreactive fibers to the bag cells. The bag cells consist of two clusters of about 400 neuroendocrine cells each that synthesize and secrete ELH and other neuroactive peptides. Confirming the results of Chiu and Strumwasser (J. Neurosci. 1:812, 1981), only the bag cell somata (BCS) and their fibers labeled for ELH. Their fibers branched profusely in the sheath and septa surrounding the ganglionic neurons, including neurons known to respond to bag cell activation. This provides a morphological substrate for ELH-mediated transmission from the bag cells to these target neurons.

IR-5HT processes were extensively branched in the sheath surrounding the BCS, while the BCS themselves were devoid of labeling. Large IR-5HT fibers apparently arising from the head ganglia coursed down the connectives and divided into several fine branches, which terminated among the BCS. IR-5HT fibers with beaded varicosities were found in a delicate array which enveloped individual bag cell somata. Since application of 5HT inhibits bag cell activity (Kaczmarek et al., PNAS 75:5200, 1978), this anatomical evidence suggests that serotonergic neurons directly inhibit the bag cells.

Branched IR-ENK processes were found in the sheath overlying the BCS, and were discernibly more numerous over the left cluster than the right one. These fibers appear to arise caudally, as only sparse enkephalin imunoreactivity was found rostrally in the connectives. There was no IR-ENK in bag cell somata, and few IR-ENK processes seen among the somata. The numerous IR-ENK ramifications in regions containing dense arborizations of bag cell processes suggest an axo-axonic relationship, although ultrastructural studies would be necessary to establish this.

IR-ENK and IR-5HT were not confined to the bag cell region. Numerous neurons and processes containing either IR-ENK or IR-5HT were observed in other parts of the ganglion. It should be possible to identify some of the neurons with combined immunocytochemical and electrophysiological techniques.

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161.19 BIOSYNTHESIS AND RELEASE OF MET-ENKEPHALIN IN TELEOST RETINA. Y. Y. T. Su. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030

The presence of enkephalin-like immunoreactive molecules and opiate-binding receptors in the retinas of several species has been reported. Recently, Djamgoz et al. (Nature, 292, 620, 1981) have demonstrated that enkephalin served as a presynaptic inhibitor to GABA containing amacrine cells in the goldfish retina. In this report a recent studies on the synthesis and release of enkephalin in goldfish and catfish retina will be presented.

The isolated fish retina was incubated in 0.2 ml of oxygenated Ringer's solution containing 40 μCi of ^{3}H-methionine and peptidase inhibitor for 30 min. The tissue was rinsed 3 times in large volumes of Ringer's solution containing peptidase inhibitor and incubated in the same unlabeled solution for another hour. The labeled tissue was homogenized in ice-cold 2 N acetic acid, boiled in a water bath for 3 min and centrifuged at 40,000 x g for 45 min. The labeled met-enkephalin presented in the supernatant was identified by immunoassay, thin layer chromatography and high performance liquid chromatography. All three methods showed that labeled enkephalin was synthesized in the tissue. A binding studies of the partially purified labeled enkephalin to mouse brain membrane preparations showed a saturable specific binding curve and this curve was completely suppresed by naloxone. All these studies demonstrated that labeled enkephalin was synthesized in the retina.

In order to study the release of enkephalin, the labeled tissues were incubated in high K^+ (60 mM) Ringer's solution, followed by several washings with normal Ringer's solution (all solutions containing peptidase inhibitor). The solutions were concentrated and assayed for enkephalin. The released enkephalin represented 8 to 10% of total enkephalin synthesized in the tissue. The release of enkephalin was completely suppresed by 5 mM Co^{+2}. This may indicate that the release is calcium dependent.

(Supported by NIH grant EY03701.)

161.20 IMMUNOREACTIVE RANATENSIN IN RAT BRAIN. T. L. O'Donohue, J. J. Pisano*, T. W. Moody, J. A. Olschowka and D. M. Jacobowitz. Lab. of Clinical Sci., NIMH; Lab. of Chemistry, NHLBI, NIGMS, NIH, Bethesda, MD 20205; Dept. of Biochemistry, George Washington University, Washington, D.C.

Ranatensin is an undecapeptide amide related in structure to bombesin and GRP-peptides which are thought to be localized in mammalian brain. Using an antisera that recognizes neither bombesin nor GRP, we have identified a ranatensin-immunoreactive peptide in brain. The peptide elutes identically to ranatensin on Sephadex G-25 chromatography but can be resolved from ranatensin into three immunoreactive peaks using reversed phase HPLC. Interestingly, one peak, but not the other two, is recognized by an antibody that specifically recognizes bombesin but not ranatensin. This peak therefore has both ranatensin and bombesin antigenic determinants but is identical to neither.

The ranatensin has been visualized in neuronal processes by immunocytochemistry in certain regions throughout the brain and the distribution determined by RIA. The distribution was quite different from that described for bombesin. Highest concentrations of ranatensin immunoreactive neurons were noted in the septum, ventral hippocampus, hypothalamus and brainstem. Terminal neuronal fields were observed in the nucleus of the tractus diagonalis, nucleus interstitialis stria terminalis, medial preoptic nucleus, median forebrain bundle, nucleus amygdala medialis, interpeduncular nucleus, nucleus parabrachialis dorsalis, nucleus tractus solitarius and the lateral reticular nucleus. The widespread distribution of the ranatensin-like neuronal processes suggests a possible role in a variety of CNS functions.

161.21 ONTOGENY OF SUBSTANCE P AND ENKEPHALIN IN THE AVIAN CILIARY GANGLION AS STUDIED BY COMBINED HPLC AND RIA. Jeffrey D. White, James E. Krause, Harvey J. Karten and Jeffrey F. McKelvy. Depts of Neurobiology and Behavior and Psychiatry, SUNY at Stony Brook, NY 11794.

The avian ciliary ganglion (CG) has proven an excellent model system for the study of cholinoceptive and cholinergic neuronal function. Recently, the co-occurrence of Substance P-like and Leu^5-enkephalin-like immunoreactivity in terminals of pre-ganglionic neurons has been described (Erichsen et al., Nature 295: 407, 1982). The present study was undertaken to determine the chemical nature of the immunoreactivities and to measure their levels during embryogenesis.

Initially, acid extracts from 20 stage 45 chick CG were subjected to High Performance Liquid Chromatographic (HPLC) analysis combined with RIA for Substance P (SP). An isocratic HPLC system was employed which separates SP, SP sulfoxide, SP free acid and SP_{5-11}. A highly specific and sensitive C-terminally directed antiserum was used in the RIA. After HPLC, a peak of immunoreactivity corresponding to the elution volume of SP was observed. For developmental studies, CG were dissected from chick embryos at stages 32, 35, 37, 40, 45, 7 days post-hatching and adult. During embryogenesis, the level of SP (per ganglion) was constant from stages 32-37 (9 pg./ganglion) and increased thereafter. By 7 days post-hatch, the level of SP had increased 4 fold to a level of 36 pg./ganglion.

Similarly, acid extracts from 20 stage 45 ganglia were subjected to gradient HPLC elution analysis which separates Met^5- and Leu^5-enkephalin, dynorphin, ß-endorphin and the amino terminal fragments of enkephalin (ENK). RIA of these HPLC fractions for Leu^5-ENK demonstrated immunoreactivity that co-eluted with Leu^5-ENK. Preliminary studies of Leu^5-ENK ontogeny indicate a peak of immunoreactivity at stages 37-40, consistent with the immunohistochemical observations of Davis, Erichsen and Karten (Soc. Neurosci. Abstr., Vol. 7, p. 400, 1981).

As a consequence of these biochemical data, studies examining the physiological role of SP and ENK in the ciliary ganglion are warranted.

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161.22 THE PRESENCE OF ANGIOTENSIN II-LIKE PEPTIDE IN CULTURED BRAIN CELLS. J.M. Kenny* and J.A. Weyhenmeyer (SPON: M. Holzwarth). College of Medicine and Neural and Behavioral Biology Program, University of Illinois, Urbana, IL 61801.

Angiotensin II (AII)-like immunoreactivity has been localized in the brains of many species including rat, monkey and human. The proposed physiological roles of the octapeptide at the level of the brain include pressor and dipsogenic responses, and the release of ADH and ACTH. Weyhenmeyer et al. have supported the existence of endogenous brain angiotensin II by demonstrating the presence of AII-like immunoreactivity in primary cultures of fetal rat brain neurons (Neurosci. Letters, 16:41,1980). Fishman et al. (Science, 214:921,1981) have shown the presence of all the components of the renin-angiotensin system in the NG108-15 neuroblastoma x glioma hybrid cells. The aim of this research was to show the de novo synthesis of AII in the NG108-15 cells and primary cultures of fetal rat brain.

For the primary cultures, brains were removed from 20 day old rat fetuses, dissociated with trypsin, and plated at $2.8x10^6$ cells/35mm Falcon plate. Primary cultures were grown for days 1,2 and 3 in Dulbecco's modified Eagle's medium with 10% fetal calf serum, 100U penicillin G, 100ug streptomycin, and 0.25ug fungizone. Cells were changed to serum-free DME supplemented with 5ug/ml of insulin and transferrin, $2x10^{-8}$M progesterone, $1x10^{-4}$M putrescine, $3x10^{-8}$M selenium, 10mg/ml L-glutamine, and the same concentrations of antibiotics. Supplemented DME was used on days 3,5 and 7 and cells were taken for experiments on day 8. NG108-15 cells were grown in DME with 12.5% horse serum and 2.5% fetal calf serum with the same antibiotic concentrations as the primary cultures for 3 days, then incubated in serum-free DME containing only antibiotics, and taken for experiments on day 4, cell density averaging $4.9x10^6$ cells/35mm plate.

On the day of the experiment, cells were incubated with 5uCi/plate ^3H proline at 37°C in an atmosphere of 10% CO_2 for 30 minutes or 1,2,3 or 4 hours. The cells were then digested and aliquots of the digest were incubated in triplicate in dilutions of 1:1, 1:10 and 1:50 with anti-AII and buffer for 36 hours at 4°C. The antibody showed 7.1% cross reactivity at 50% displacement with AI. The antigen-antibody complex was precipitated with dextran and charcoal and centrifugation and counted in a scintillation counter. Upon comparison with a standard curve developed for the RIA, preliminary evidence indicates the presence of pg levels of AII-like peptide per mg of protein in the cellular digest.

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162.1 MU, KAPPA, AND OTHER OPIOID COMPOUNDS IN MOUSE TASTE AVERSION EXPERIMENTS. Peggy J. K. Dobry, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Administration of a novel drug after a novel-tasting substance can lead to decreased ingestion of the substance when it is offered later. This "taste aversion" can be conditioned in animals both by drugs which humans self-administer and by drugs which humans avoid, and even by drugs which animals self-administer intravenously.

On Day 1, water-deprived CF-1 female mice were offered 5% sucrose solution for exactly 15 minutes. Immediately, mice were injected subcutaneously with saline or drug. On Day 2, the procedure was repeated. For the next 24 hours, mice had free access to water. On Day 4, in the Test Session, water-deprived mice were offered sucrose solution for 10 minutes (in addition, water was available in the Test Session, but its consumption was not measured). Groups were compared statistically by the Mann-Whitney U test (one-tailed test for drug-induced decreases in fluid consumption). Note that the Test Session occurred 48 hours after the last drug injection.

Agonists and antagonists of the mu receptor cause powerful dose-related taste aversions. The mg/kg dose causing 50% suppression of drinking is: morphine sulfate, 3.9; methadone, 2.3; pentazocine (lactate), 5.4; butorphanol tartrate, 15; cyclazocine, 1.6. Similar results were obtained for the enkephalin analog FK-33824 (0.24).

Primarily-kappa opioids did not cause taste aversion; bremazocine (0.3-3 mg/kg), ketocyclazocine (1-10 mg/kg), ethylketocyclazocine methanesulfonate (3-30 mg/kg), U-50,488H (3-30 mg/kg).

The sigma agonist SKF-10047 caused a dose-related taste aversion with 50% suppression at 6.6 mg/kg.

Naloxone hydrochloride did not cause taste aversion at 1 and 3 mg/kg, which are effective narcotic-antagonist doses.

Nalorphine hydrochloride is a special case, since 1 and 3 mg/kg caused a significant increase in fluid consumption. This increase was seen even with 5 days between the final nalorphine injection and the Test Session -- 4 of these days with water ad libitum. Hence, it is highly unlikely that the increased drinking is due to some non-specific dehydration. Increased drinking was not seen in rats in a similar test.

The differences in taste aversion predict that kappa agonists will have entirely different subjective effects from those of mu, sigma, or delta agonists.

162.2 MORPHINE MICROINJECTED INTO NUCLEUS RETICULARIS PARAGIGANTOCELLULARIS REDUCES ACTIVITY OF NEURONS IN NUCLEUS RAPHE MAGNUS. Mary M. Heinricher and J. Peter Rosenfeld. Depts. of Psychology and Neurobiology & Physiology, Cresap Neuroscience Laboratory, Northwestern University, Evanston, Ill. 60201.

Although microinjection of morphine into, or electrical stimulation of the nucleus reticularis paragigantocellularis (PGC) results in behaviorally-defined analgesia, the neural substrate of this effect has yet to be determined. It has been suggested that the analgesia is mediated through the nucleus raphe magnus (NRM) (Azami, et al., J. Physiol., 306: 16-17P, 1980). The present experiment was designed to investigate the effect of PGC morphine microinjections on unit activity in NRM.

Rats were anesthetized with urethane (1.2 g/kg) and a 25 gauge guide cannula implanted above PGC. NRM unit activity was recorded using a glass micropipette. The firing rate of NRM units ranged from 1 to 44 Hz. The great majority of units responded to noxious pinch. After a unit was isolated, morphine sulfate (1 µg in 0.5 µL) was infused into PGC through a 31 gauge injector cannula over the course of 4 minutes. Only 1 cell was used per animal.

The predominant effect of PGC morphine microinjections was suppression of spontaneous activity. Over half of the units showed a reduction of firing rate of at least 25%, while most of the remaining cells showed a less pronounced suppression. This effect was antagonised by systemic naloxone (1 mg/kg) in 66% of the cases in which it was attempted. Only one cell showed a substantial increase (71%) in firing rate after PGC morphine, and this effect was not reversed by naloxone. These results suggest that the analgesic effect of PGC morphine microinjections is not mediated by an increase in descending inhibition from NRM.
(Supported by grants GM23696 and T32-MH16097.)

162.3 HALOPERIDOL CATALEPSY IS ANTAGONIZED BY A HIGH DOSE OF MORPHINE IN THE C57BL/6J MOUSE. K. E. Stevens and G. A. Mickley. Department of Behavioral Sciences and Leadership, USAF Academy, CO 80840.

In the rat, and in most other species, a sufficiently large dose of morphine produces hypokinesia and catatonia. Similar behaviors can be observed after an injection of a dopamine receptor blocking agent (e.g., haloperidol). These and a variety of biochemical observations have led many to suggest that morphine might also produce some of its behavioral effects by inhibiting dopamine receptor activity as well (H. Lal, Life Sci. 17, 483-496, 1975). The data reported here highlight some behavioral differences evoked by haloperidol and morphine in the C57BL/6J mouse and support the hypothesis that there may be different mechanisms which mediate the behavioral changes produced by the drugs (H. Lal, et.al., Life Sci. 17, 29-34, 1975).

Pre-drug baseline measures of catalepsy and spontaneous locomotor activity were recorded from 60 male C57BL/6J mice. Some of these animals received a first injection of 2 mg/kg haloperidol. The catalepsy and hypokinesia produced by the haloperidol was later challenged with either a 30 or 60 mg/kg injection of morphine. Control animals received either saline or haloperidol as their first injection then either morphine or saline as a challenge. Post-drug catalepsy measures were taken 75 minutes after the first injection and then 60 minutes after the second injection. Spontaneous locomotion was also recorded after administration of each of the drugs.

C57BL/6J mice exhibited quite different behavioral responses to the two drugs. Haloperidol produced catalepsy and hypokinesia in these mice. However, when morphine was administered (after a control saline injection) it produced a stereotypic locomotor hyperactivity and no catalepsy. When morphine was given as a challenge to haloperidol's behavioral effects it effectively countered the catalepsy ($p < .05$, Kruskal-Wallis). Interestingly, however, morphine did not significantly increase the locomotion of these mice ($p > .05$, Kruskal-Wallis).

Although opiate actions may be in some way, related to the inactivity of dopamine receptors, morphine's reversal of haloperidol catalepsy suggests that perhaps the neurochemical actions of morphine and haloperidol are not identical in this mouse. In additon, since morphine did not reverse the hypokinesia of haloperidol-treated mice, but did counter the catalepsy, it may be the case that the neurochemical substrates of these two behaviors are, in some ways, different.

This research was supported by Defense Nuclear Agency.

162.4 REINSTATEMENT OF HEROIN-REINFORCED RESPONDING IN THE RAT BY CENTRAL IMPLANTS OF MORPHINE IN THE VENTRAL TEGMENTAL AREA. J. Stewart. Dept. of Psychology, Concordia Univ., Montreal, Canada H3G 1M8.

Non-contingent intravenous 'priming' injections of heroin or morphine reinstate heroin-reinforced responding after a period of extinction in rats trained to self-administer 100 µg/kg/infusion intravenously. Rats similarly trained were implanted with intracerebral cannulae directed at either the ventral tegmental area (VTA), the caudate nucleus (CN), or the periventricular grey (PVG). Each rat had cannulae placed at two of these sites. Following extinction of lever-pressing for heroin, crystalline morphine HCl applied via a 28-guage stainless steel tube into the VTA reinstated heroin-reinforced responding for up to 3 hours in spite of continued extinction conditions. No effect of morphine was seen at the other two sites. Pretreatment i.p. with 2 mg/kg naltrexone HCl 30 min prior to testing blocked the effect of the morphine implant in the VTA. These data add support to the view that the positive incentive properties of heroin and morphine are mediated via the mesolimbic dopamine system.
(Supported by a grant from the Medical Research Council of Canada MA 6678.)

162.5 FAILURE TO BLOCK THE OPIATE EFFECTS OF ETONITAZINE WITH NALTREXONE IN RATS. M.R. Lynch, J. H. Porter* and D. N. Johnson. Dept. of Psychology, Va. Commonwealth Univ., Richmond, VA 23284.

Naltrexone blockade of the opiate effects of oral etonitazine was attempted in a choice situation with .01 mg/ml quinine in order to equate drug and placebo solutions on the basis of palatability. Ten male albino rats were provided with 5 μg/ml etonitazine HCl solutions and the quinine for 24 hrs. each day, with bottle positions counterbalanced over days to control for position preferences. Naltrexone was administered i.p., once per day, in a dose of 3 mg/kg. After six days access to the drug and placebo solutions, 3 of ten animals began showing precipitated withdrawal following antagonist injections. This number increased to 6 rats on day seven and these rats continued to show precipitated withdrawal over the remaining 4 days of naltrexone administration. Intakes of the etonitazine solution ranged from negligible amounts to as much as 115 mls, representing μg/kg/day doses of 28 to 825 over the last three days of choice-testing. Rats exhibiting withdrawal drank 412 to 825 μg/kg/day. Analgesic testing to examine cross-tolerance to 5 mg/kg morphine was conducted 24 and 48 hrs. after the last naltrexone injection, with animals remaining on choice tests over these 48 hrs. After antagonist action had disipated (48 hrs.), a significant difference in post-morphine tail flick latency was noted between animals consuming low doses of drug ($\bar{X}$ = 167 μg/kg/day) and those five with the highest intake ($\bar{X}$ = 614). While no spontaneous withdrawal symptoms were noted over six days following removal of choice-test solutions, daily water intake dropped to approximately one-half of previous fluid consumption and gradually recovered over the six days. Body weights also dropped over the first 96 hrs. of this abstinence period and then recovered. These six days were followed by 12 more days of continued choice-testing, but with daily injections of saline only. Repeated cross-tolerance testing was conducted after 12 days of this testing, with identical results to the 48 hr. test conducted after daily naltrexone injections. Water intakes over six more days of abstinence, showed a similar decrease and recovery as in the previous opiate-free period, and body weights dropped from a mean of 450g to 410g over the first 24 hours and then gradually recovered. The results indicated a failure to block opiate effects of orally ingested etonitazine, when intake exceeds 350 μg/kg/day.

162.6 SUBCUTANEOUS INFUSION OF MORPHINE BY OSMOTIC MINIPUMP: TOLERANCE AND DEPENDENCE WITHOUT ANALGESIA. D.H. Malin, K.E. Peek*, K. Freeman*, W.R. Mills* and L.S. Neal*. Univ. of Houston at Clear Lake City, Houston, TX 77058.

Infusion of opiates via osmotic minipumps offers certain advantages in comparison with pellet implantation: steady, precise infusion rate and ease of removal. Previous authors (Wei and Loh, 1976; Simmons, et al., 1978; Lange, et al., 1979) have produced morphine tolerance or dependence in the rat through osmotic minipumps connected to intraventricular cannulas. We now report that tolerance and dependence can be achieved by the much simpler procedure of s.c. minipump implantation.

Subjects were female Sprague-Dawley rats ($\bar{x}$ = 275 g). Alzet osmotic minipumps (model # 2001) were filled with a 50 mg/ml morphine sulfate solution or with distilled water alone. Minipumps were inserted s.c. through a 2-cm incision in the scapular region of rats under light anesthesia. The incision was closed by 2 skin clips. This procedure required less than 3 minutes. It has been demonstrated (Theeuwes and Yum, 1976) that the minipump delivers 1 μl/hr continuously for at least 7 days. Thus, each rat received only .014 mg/kg/hr. Not surprisingly, this minute dose failed to produce analgesia on the tail-flick test. In fact, morphine-infused animals showed significantly lower flick latencies than water-infused controls at intervals ranging from 12 hours to 6 days post-implantation.

Nevertheless, rats demonstrated considerable tolerance to morphine after 7 days of infusion. Four morphine-infused rats showed significantly less increase of tail-flick latency in response to 4 mg/kg s.c. morphine as compared with water-infused controls.

In addition, rats demonstrated morphine dependence following 7 days of infusion, as evidenced by naloxone-precipitated abstinence symptoms. In response to 2 mg/kg s.c., 4 morphine-infused rats showed significantly more writhes, wet-dog shakes, dyspnea, and teeth-chattering as compared to 4 water-infused animals. Another experiment demonstrated spontaneous withdrawal. Morphine and water pumps were removed from rats under light anesthesia 7 days after implantation. Five rats formerly implanted with morphine pumps showed significantly more abstinence-like symptoms (wet-dog shakes, head shakes, scratches, dyspnea) than 5 animals formerly implanted with water pumps. This difference persisted for 3 days after pump removal.

Dependence formation by morphine infusion could not be accounted for by change in brain tissue endorphin levels. RIA for Met-enkephalin revealed 632 ± 22 ng/g (mean ± SEM) in 5 rats infused with morphine for 7 days and 638 ± 77 in rats infused with distilled water for 7 days. RIA for beta-endorphin revealed 189 ± 9 ng/g in morphine-infused brains and 193 ± 11 in water-infused brains. These differences were not significant.

162.7 INTRACEREBRAL INJECTION OF NALOXONE IN THE REGION OF THE NUCLEUS TRACTUS SOLITARIUS: EFFECT ON EEG CHANGES INDUCED BY SYSTEMIC MORPHINE M. Kelly,* J.D. Bronzino, N. Oley, C. Cordova,* and P.J. Morgane (SPON: K. Morest). Trinity College, Hartford, Connecticut, 06106.

Recent evidence has indicated that the nucleus of the solitary tract and area postrema (NTS/AP) region of the dorsomedial medulla plays a role in pain suppression. For example, the region of NTS/AP has been found to be rich in opiate receptors and to be involved in pain modulation when stimulated with noradrenlin. We recently reported that microinjections of morphine in the region of the NTS/AP produced analgesia using the traditional tail-flick test (Brain Research, 236:511-515, 1982) and the EEG and behavioral effect of systemic administration of morphine (EEG Clin. Neurophysiol., 53:14-26, 1982). In the present study we set out to determine whether microinjections of naloxone, an opiate antagonist, applied directly into the region of the NTS will prevent the EEG effects of subsequent systemic injection of morphine. Male Sprague-Dawley retired breeder rats had microinjections (0.1 μl) of Naloxone at various doses (i.e., 1 μg, and 2.5 μg) or Ringer's solution directly injected into the region of the NTS via a 28 gauge cannula system. Five minutes following the intracerebral injection of naloxone, the animal was given an injection of 30 mg/kg morphine sulphate i.p. Intracerebral injection of naloxone into the region of the NTS was effective in significantly decreasing the EEG synchronizing effect of systemic administration of morphine. These microinjections had a major impact upon the percent of animals reaching High Voltage Low Frequency (HVLF) activity and the amount of time spent in HVLF activity. In our earlier work (Society of Neuroscience p.450, 1981), we showed that 30 and 10 μg injections of naloxone into the region of the NTS produced significant changes in the EEG synchrony (HVLF) that normally follow a morphine injection. These early results, however, failed to show a clear differentiation between animals whose cannula were within the NTS and those slightly out of this region. With doses of 2.5 and 1.0 μg a definite histological specificity emerges. At 1.0 μg of naloxone, for example, the control placement outside NTS produced results similar to those obtained with sham and Ringer's control while the placements in the NTS resemble the effects produced by higher doses of naloxone in the NTS. These results indicate that the primary effect of naloxone into the region of the NTS is upon morphine-induced EEG synchronization and suggests that the region of the NTS is involved in this morphine-induced EEG effect. (Supported by NIH Grant GM 27226-01).

162.8 DISRUPTION OF MATERNAL BEHAVIOR AFTER SYSTEMIC OR CENTRAL ADMINISTRATION OF MORPHINE IN RATS. B. S. Rubin*, C. T. Grimm* and R. S. Bridges. Lab. of Human Reprod. and Reprod. Biol., Harvard Medical School, Boston, MA 02115.

Opiate immunoreactivity in the hypothalamus as well as pain thresholds change during pregnancy and lactation in rats. In the present study we examined the possibility that opiates may be involved in regulating maternal responsiveness. In experiment #1 the effects of systemic treatment with morphine (M) and the opiate antagonist naloxone (N) on the onset and maintenance of maternal behavior were measured. Twenty-five primigravid rats had their pregnancies terminated by ovariectomy plus hysterectomy (OH) on day 17 of gestation. After surgery subjects were injected with M (5mg/kg), M plus N (0.5mg/kg) or saline (S). Animals were thereafter injected with these substances daily from 0900-1000 h. Behavioral testing was initiated 1 hr after injections beginning 24 hr after OH. Pup-oriented and nest building responses were scored each day during a 1 hr test. M treatment significantly disrupted the onset of maternal behavior, whereas concurrent administration of N to M-treated rats prevented the disruptive effects of M alone. Latencies to show full maternal behavior to foster young were 5.0 days for M, 0.4 days for S and 0.5 days for M plus N rats. M treatment alone also diminished the quality of maternal responsiveness in a T-maze test, but did not reduce activity levels in an open field test. In experiment #2 we attempted to localize morphine's disruptive action in the CNS by applying M directly to the medial preoptic area (MPOA), an area involved in the mediation of maternal behavior, or the ventromedial nucleus (VMN) of the hypothalamus. M-filled cannulae were lowered into the brains of 26 ovx, estradiol-primed, pup-induced maternal virgins. Subjects were tested for maternal responsiveness both after M-filled and after blank cannulae were lowered into place. Eleven of 13 females with M-filled cannulae in the MPOA showed a dramatic disruption of maternal responsiveness during the 1 hr test session; however, these same 11 subjects exhibited a normal maternal response when blank cannulae were lowered into the same sites. In contrast, only 3 of 13 females exhibited some deficit in maternal behavior after M-filled cannulae were placed in the VMN, and the deficit was less severe. The results of activity tests revealed that direct application of M to the MPOA did not alter activity levels, whereas M application to the VMN suppressed open field activity. In summary, these data indicate that morphine can act centrally to modulate the expression of maternal behavior and thereby suggest a possible role for endogenous opiates in the mediation of maternal responsiveness. Supported by NSF Grant #BNS 80-14670 and March of Dimes Birth Defects Foundation Grant #BOC 5-311 awarded to RSB.

162.9 OPIOID MODULATION OF STIMULATION-INDUCED FEEDING: COMPARISON OF CENTRALLY AND PERIPHERALLY ACTIVE COMPOUNDS. K. D. Carr, K. A. Bonnet and E. J. Simon. Departments of Psychiatry and Pharmacology, New York University School of Medicine, New York, NY 10016.

Recent evidence indicates that the endogenous opioids are involved in the regulation of food intake. Several laboratories have reported that naloxone, the opiate antagonist, suppresses eating in food-deprived animals. While opioid peptides and opiate receptors are indeed located in regions of the CNS that are involved in mechanisms of hunger, taste and reinforcement, they are also located in peripheral tissues such as the gastrointestinal tract, adrenal medulla and pancreas where opioid activity could affect feeding. The purpose of the present study was to discriminate between peripheral and central opioid activity in the acute regulation of feeding behavior. Frequency threshold for feeding induced by electrical stimulation of the lateral hypothalamus was examined in rats after administration of opioid agonist and antagonist compounds that show differential penetrability of the blood-brain barrier. There is evidence that, unlike naloxone and morphine, quarternary naloxone and the agonist loperamide do not cross the blood-brain barrier. We therefore determined stimulation-induced feeding threshold prior to and 20 min after subcutaneous injection of each of these four compounds.

Naloxone elevated stimulation-induced feeding threshold in a dose-related manner (0.2 - 5.0 mg/kg) while quarternary naloxone (2.0 - 10.0 mg/kg) did not. Progressive increases in naloxone's threshold-elevating action over the course of a session suggest an effect on post-ingestive events, possibly a reduction in the reinforcement of consummatory behavior. Morphine sulfate reduced stimulation-induced feeding threshold at low doses (e.g. 1.25 mg/kg) but elevated threshold at higher doses (2.5, 5.0 mg/kg). Loperamide either had no effect (0.0625, 0.125 mg/kg) or elevated threshold (0.25 mg/kg). The threshold-elevating effect of loperamide was blocked by pretreatment with quarternary naloxone.

These results indicate that the anorectic action of naloxone is due to blockade of receptors in the CNS and that central opioid activity may play a role in the reinforcement of consummatory behavior. The suppressive effect of peripheral opioid activity on feeding may be due to the inhibition of gastrointestinal motility.

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162.10 LIMBIC ACETYLCHOLINE TURNOVER RATES CORRELATED WITH MORPHINE-SEEKING BEHAVIOR, J.E. Smith, C. Co* and J.D. Lane. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Opiate self-administration is maintained by the reinforcing properties of these drugs. Acetylcholine releasing neurons may be involved in these reinforcement processes since disruption of cholinergic neuronal activity with atropine attenuates intravenous morphine self-administration (Davis and Smith, Life Sci. 16, 237, 1975). This study was initiated to directly assess the involvement of cholinergic neurons in opiate reinforcement. The turnover rates of acetylcholine (ACh-TO) were measured in the frontal cortex (FC), pyriform cortex (PC), caudate nucleus (CN), diagonal band (DB) and hippocampus (Hipp) of rats intravenously self-administering morphine (I) and in yoked-morphine infused (II) and yoked-vehicle infused (III) littermates. Fourteen litters of three (conditions I-III) male F-344 rats were implanted with chronic jugular catheters and two littermates in each group made physically dependent upon morphine with hourly infusions. One of the dependent rats was then allowed to self-administer morphine (10 mg/kg) by lever pressing. The other two littermates in each group received simultaneous yoked infusions. After stable baselines of self-administration were obtained, an average interinjection interval was calculated for each self-administering animal. At 5 min (N=7 litters) and 10 min (N=7 litters) prior to a predicted self-infusion, 0.5 mCi of [^{3}H]-choline were injected through the jugular catheter and the litter sacrificed by immersion in liquid nitrogen at the predicted self-administration time. The brains were removed at -20°C and the areas of interest dissected from serial coronal sections at -20°C. Content and turnover rates were determined with modifications of previously described procedures (Shea and Aprison, Anal. Biochm. 35, 136, 1969; Fiegenson and Saelens. Biochem Pharmacol. 18, 1479, 1969). Passive morphine infusion (comparing Group II and III) resulted in a decrease in ACh-TO in the CN and significant increases in the DB and Hipp. Morphine self-administration (comparing groups I and II) resulted in significant increases in ACH-TO in the FC and decreases in the PC, CN and Hipp. The changes in ACh-TO in the FC, CN and Hipp in the self-administering rats are consistent with the proposed involvement of a FC-CN and a Hipp loop in opiate reinforcement [suggested by prior investigations of the turnover rates of biogenic amine and amino acid neurotransmitters in self-administering rats (Smith et al, Nature 287, 152, 1980; Pharmac. Biochem. Behav. 16, 509, 1982)]. (Supported in part by USPHS Grant No. DA-01999-04).

162.11 DIFFERENTIAL RESPONSIVITY TO MORPHINE IN MONOSODIUM GLUTAMATE TREATED RATS. N. Nicotera, D. Badillo-Martinez, P. Butler*, A.L. Kirchgessner*, V. Sikorszky* and R.J. Bodnar. Dept. of Psychology, Queens College, C.U.N.Y., Flushing, NY 11367.

The medial-basal hypothalamus appears to be involved in pain perception given its concentration of brain B-endorphin and ACTH cells as well as the observed deficits in morphine analgesia following its destruction by neonatal treatment with monosodium glutamate (MSG). However, this brain area may mediate through opioids other behavioral responses as well. Therefore, MSG-induced changes in opiate analgesia must be understood in terms of selective or general behavioral deficits. To this end, neonatal rats received either a high dose (HMSG: 2g/kg on days 2 & 4; 4g/kg on days 6, 8 & 10), a low dose (LMSG: 1 g/kg on days 2 & 4; 2g/kg on days 6, 8 & 10) or a saline vehicle (SAL). Beginning at 80 days of age, flinch-jump and hot plate thresholds were measured following a dose range of morphine. While HMSG rats displayed increased jump thresholds following the 2.5 mg/kg morphine dose, they exhibited an attenuated analgesia following 5 and 10 mg/kg doses. Conversely, HMSG rats showed significantly longer hot-plate latencies following the 10 mg/kg morphine dose with analgesia persisting over time. Hypoactivity induced by the 10 mg/kg morphine dose failed to dissociate across groups, but hyperactivity following the 2.5 mg/kg dose was present in SAL and LMSG, but not in HMSG, rats.

Flinch-jump thresholds, hot-plate latencies, body temperature and body weight were measured every fourth day following daily morphine (15 mg/kg) injections over 14 days. Maximal morphine analgesia on jump thresholds occurred on day 1 for SAL rats, on days 1 and 5 for LMSG rats and on day 5 for HMSG rats. Gender-specific effects were also apparent on this measure with males exhibiting more pronounced differences across groups. By contrast, repeated morphine injections altered hot-plate latencies and hyperthermia similarly across groups. The chronic morphine injections resulted in more marked weight loss in HMSG rats. Therefore, the analgesic and locomotor changes noted in HMSG rats following acute morphine appear to be dependent upon dose, pain test and gender. Moreover, HMSG rats appear to display a delayed maximal analgesic response following repeated morphine injections with pain test and gender as critical variables. These data indicate that MSG-induced loss of the B-endorphin/ACTH system, as well as other pertinent hypothalamic neuromodulators, produce pronounced, yet selective, effects upon an animal's analgesic, locomotor and weight control responses following acute or chronic opiate administration. Supported by NIH GRSG 5-S05-RR-07064.

162.12 EFFECTS OF KAINIC ACID LESIONS OF THE NUCLEUS ACCUMBENS ON RESPONDING MAINTAINED BY A CONCURRENT FOOD, WATER AND MORPHINE SCHEDULE, S.I. Dworkin, G.F. Guerin, J.D. Lane, D.R. Cherek and J.E. Smith, Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Results from several studies suggest that operant behavior maintained by different reinforcers may share specific neuronal systems that mediate reinforcement processes. The nucleus accumbens has been shown to support both intracranial self-stimulation and opiate self-administration,while lesions decrease responding maintained by cocaine self-administration. This study assessed the effects of lesions of the nucleus accumbens on concurrent food, water and morphine reinforcement.

Male Fisher F-344 rats were trained to lever press on separate retractable levers on a non-reversable choice schedule involving food and water presentation. The subjects were implanted with bilateral intracranial injection cannulae into the nucleus accumbens and with a jugular catheter. The three reinforcers were each available with 24-hour access on FR 10 schedules with a 100 sec limited hold contengency and each reinforcement or elapsed limited hold was followed by a 30 sec time out. Chemical lesions of the nucleus accumbens were then produced by bilateral microinjections of kainic acid while control animals received microinjections of saline.

Comparable rates and temporal patterns of responding were maintained by the three reinforcers. Between 7 and 20 morphine infusions were delivered each day. The number of food and water presentation was 10 to 15 fold larger. Most of the food and water was taken during the subject's dark cycle (between 5 am to 5 pm). Morphine infusions were more evenly distributed over 24 hours. Kainic acid lesions of the nucleus accumbens resulted in an initial decrease in responding maintained by all three reinforcers. The total number of food and water presentations subsequently increased to values observed before the lesion while morphine infusions decreased to about 50 percent. Food and water presentations were distributed during both light and dark cycles. However, morphine infusions were no longer evenly distributed over the 24-hour period.

The effects of extinction were studied with pre-lesion subjects to further assess the behavioral effects of the lesions. Changing the food schedule to extinction resulted in large increases in morphine intake with a concomitant decrease in water intake while extinction of responding maintained by morphine decreased food and water responding. The effects of the lesion on responding maintained by the three reinforcers were not similar to the effects of extinction. (Supported in part by USPHS Grant DA-01999-04).

162.13 REVERSAL OF MORPHINE (M)-INDUCED CATALEPSY IN RATS BY QUATERNARY NARCOTIC ANTAGONISTS. D.R. Brown, M.J. Robertson*, L.I. Goldberg* Dept. Pharmacology, University of Chicago, Chicago, IL 60637

The pure narcotic antagonists, naloxone (Nx) and naltrexone (Ntx), are useful pharmacological tools in opiate research; however, their value in determining sites of opiate action is precluded by their rapid diffusion across the blood-brain barrier. Their quaternary derivatives, naloxone methobromide (NxMeBr) and naltrexone methobromide (NtxMeBr) may be less permeable to the blood-brain barrier due to their limited lipid solubility. We evaluated the abilities of NxMeBr and NtxMeBr as well as Nx and Ntx, given peripherally, to reverse M-induced catalepsy in rats; this opiate-induced rigid immobility is presumably mediated at CNS sites. Moreover, the action of NtxMeBr was assessed after intracerebroventricular (i.cv.) administration. Male Sprague-Dawley rats (300-350 g) were given M (20 mg/kg, base; s.c.) and tested for catalepsy at 30 min intervals from 30-240 min after injection. Two groups of rats received random doses of Nx and NxMeBr or Ntx and NtxMeBr, respectively, and saline administered s.c. 40 min following M. A third group of rats with chronic cannulae implanted in the right cerebral ventricle were injected with random doses of NtxMeBr (0.1-1.0 µg/5 µl) or saline (5 µl), i.cv. 70 min after M administration. Duration of catalepsy was determined by placing animals' forepaws over a horizontal bar 10.5 cm high; catalepsy was measured with a 300 sec cutoff time. Rats given saline after M injection remained fully cataleptic for over 120 min. The peripheral dose of each drug necessary to shorten catalepsy duration to 150 sec (ED_{50}) appears below at 60, 90, and 120 min after M administration:

	ED_{50}* (mg/kg)		
	60	90	120
Nx	0.0007	0.018	0.017
NxMeBr	1.06	1.36	1.27
Ntx	0.007	0.008	0.002
NtxMeBr	20.9	10.8	2.4

*Based on least squares regression of means; n = 5-9 animals/point

NtxMeBr had an ED_{50}=0.001 mg/kg at 90 min post-M when administered i.cv. These results indicate that 1) NxMeBr may penetrate the blood-brain barrier but is less potent than Nx; 2) NtxMeBr, given s.c. is initially impermeable to the CNS although its potency increases with time suggesting either slow diffusion into the brain or the formation of active, lipophilic metabolites; 3) NtxMeBr, given i.cv. is 21,000-fold more potent in reversing catalepsy than it is upon s.c. injection. Thus, NtxMeBr may be useful in defining sites of opiate action and in therapeutically blocking undesirable peripheral effects of opiate analgesics.

162.14 NALOXONE INCREASES SOCIAL NEED. L. Normansell*, S. Siviy* K. White* and J. Panksepp Dept. of Psych., Bowling Green State Univ., Bowling Green, OH 43403

In past work we have demonstrated that brain opioids inhibit emotions resulting from social separation while blockade of opiate receptors increases separation distress. Accordingly, the brain opioid theory of social affect predicts that opiate receptor blocking agents should increase social motivation. However, we have repeatedly observed naloxone (1-10 mg/kg) to reduce social play in young rats. This observation could be deemed inconsistent with the above theory, but it is the expected outcome from the perspective that an essential condition for vigorous play is a state of social comfort. To further determine whether naloxone does in fact increase social motivation, young rats were tested in a variety of discrete situations with social rewards.

Opioid blockade (naloxone 1 mg/kg) increased the tendency of 15-20 day old rats deprived of both food and home overnight to choose home rather than food reward. Similarly, 10-13 day old rat pups treated with naloxone (10 mg/kg) ran from clean bedding to dirty home bedding twice as fast as controls, while 16 day old animals given a choice between dirty home bedding on one side of a test chamber and their anesthetized mother on the other, latched a nipple in a third of the time following naloxone (1 mg/kg) compared to saline. However, juvenile rats (25-40 days of age), tested for play motivation in a spatial learning situation (U-maze), seemed socially timid following naloxone, exhibiting slow entry into the play chamber (average latency: naloxone (1 mg/kg)--4.3 sec, saline--1.9 sec). During 9 days of extinction testing, naloxone treated animals also ran much slower than control or morphine treated ones (mean running speed for last eight days of extinction: saline--22.6 sec; morphine (1 mg/kg)--11.7 sec; naloxone--81.3 sec) and made fewer correct responses (saline--57%, morphine--65%, naloxone--20% correct).

Taken together, these results affirm that naloxone increases social need. Accordingly, the reduction of play following naloxone may be due to a reduced ability of an animal to elaborate a brain state of social comfort which normally promotes energetic and competitive social interactions.

162.15 PHARMACOLOGICAL SEPARATION OF AGGRESSION FROM OTHER SYMPTOMS OF MORPHINE WITHDRAWAL. K.M. Kantak and K.A. Miczek. Dept. Psych., Tufts Univ., Medford, MA 02155.

Withdrawal from morphine engenders a characteristic behavioral syndrome. Naloxone administration readily precipitates this syndrome. Heightened aggressive behavior has also been associated with morphine withdrawal. The following experiments were conducted to determine the mechanisms which might mediate the aggressive and other symptoms of morphine withdrawal. Initially, morphine withdrawal aggression was investigated in mice with or without fighting experience. 75mg morphine or placebo pellets were implanted in resident mice who showed attack and threat behavior toward intruder animals. In a second group non-experienced resident mice were given morphine or placebo pellets. Parellel experiments were performed in intruder animals who showed defensive and escape behaviors. After tolerance to morphine was established, naloxone HCl (1 or 10 mg/kg, i.p.) was administered, and withdrawal, including changes in aggression, was evaluated immediately post-injection. In other animals, the pellets were removed and behavioral measures were taken 48 hrs later. Morphine withdrawal aggression was observed following morphine pellet removal in non-experienced animals. An increase in biting attacks was observed in both resident and intruder mice. In contrast, naloxone decreased attack in non-experienced and experienced resident mice and increased escapes and vocalizations in non-experienced and experienced intruder mice. These effects of naloxone were observed in both morphine and placebo treated mice. The anti-aggressive action of naloxone might not be related to withdrawal from the µ-opiate receptor while the aggression enhancing action of morphine pellet removal might be. Since µ-opiate receptors may influence catecholamine functioning, a second series of experiments was conducted in which non-experienced residents were withdrawn from morphine by pellet removal and tested for aggression against intruders 48 hr later with various doses of apomorphine HCl (0.01-1.0 mg/kg, i.p.) and clonidine HCl (0.1 and 0.25 mg/kg, s.c.). At doses of apomorphine which primarily act on presynaptic dopamine receptors (0.01, 0.03 and 0.1 mg/kg) morphine withdrawal aggression and jumping behavior were not affected. Hyperthermia was produced by 0.01 and 0.03 mg/kg. With 1.0 mg/kg of apomorphine, which primarily acts on post-synaptic dopamine receptor, morphine withdrawal aggression and jumping were suppressed. This was accompanied by hypothermia. Clonidine a presynaptic α-noradrenergic agonist, also suppressed attacks at 0.1 and 0.25 mg/kg, but caused an increase in withdrawal jumping. A slight hypothermia at both clonidine doses was measured. This suggests that morphine withdrawal aggression can be viewed independently from other morphine withdrawal symptoms.

162.16 DISCRIMINATIVE AND REINFORCING STIMULUS PROPERTIES OF STEREOISOMERS OF CYCLAZOCINE. Barbara Lord Slifer*, Robert L. Balster* and W.D. Rodes* (SPON: M.J. Kallman). Pharmacology Department, Medical College of Virginia, Richmond, VA 23298/

Cyclazocine (C), a benzomorphan with sigma-receptor agonist activity has been shown to be similar to the prototype sigma agonist N-allylnormetazocine (NANM) and the dissociative anesthetic phencyclidine (PCP). For example: C, NANM and PCP all produce psychotomimetic effects in humans (Domino & Luby, 1981; Keats & Telford, 1964) and result in a similar profile of effects in the chronic spinal dog (Gilbert & Martin, 1976; Vaupel & Jasinski, 1979). Recent binding studies with C in rat brain (Zukin & Zukin, 1981) have shown multiple sites with displacement at a low affinity site by PCP and NANM but not naloxone or morphine. In spite of such demonstrated similarities, certain differences still exist. While PCP is an effective reinforcer in maintaining self-administration behavior, the psychotomimetic benzomorphans are not self-administered by animals. Recent evidence has indicated that the (+)-isomer of NANM is more specific than the (-)-isomer for producing the PCP-like discriminative- and reinforcing-stimulus effects of this compound (Brady et al., 1981; Slifer and Balster, 1982), and similar isomeric separation of discriminative stimulus effects of C have been shown in squirrel monkeys in previous research in this laboratory. We report here on the discriminiative stimulus effects in rats and the self-administration by rhesus monkeys of the racemic mixture and the pure stereoisomers of cyclazocine.

Rats were trained on a PCP-SALINE 2-lever discrimination task for food pellets on a FR-32 schedule of reinforcement. Training was done on a double alternation schedule and test sessions consisted of 2-min extinction periods during which various doses of PCP, (±)-C and the pure (+)- and (-)-isomers of C were tested. PCP (0.3 - 3.0 mg/kg) and (+)-C (1.0 - 30.0 mg/kg) produced dose-related drug-lever appropriate responding. Neither the (-)-isomer (0.03 - 1.0 mg/kg) nor (±)-C (0.1 - 3.0 mg/kg) resulted in over 40% PCP-lever responding at doses which markedly suppressed response rates. Rhesus monkeys were trained to self-administer (SA) i.v. cocaine (50 µg/kg/inj) by lever pressing on a FR-10 schedule of reinforcement. Doses of cyclazocine ((±), (+), (-)) or saline were substituted for cocaine for 4 consecutive daily sessions. Substitution of the racemate (0.1 - 3.0 µg/kg/inj) or (-)-C (0.03-3.0 µg/kg/inj) did not result in responding above saline levels at any dose tested while the (+)-isomer maintained rates of self-administration which were well above saline SA rates at the 10.0 µg/kg/inj dose in all monkeys tested. It appears that the PCP-like stimulus properties of cyclazocine, like NANM, are revealed in the (+)-isomer in both rats and monkeys. (Supported by grants DA-01442 and DA-05193).

163.1 SODIUM HUNGER AND THE VOLUNTARY INTAKE OF ETHANOL. L. A. Grupp. Dept. of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

A number of reports, both recent and in the recent past, point to the involvement of sodium in choice and voluntary consumption of ethanol. Iida (Jap. J. Pharmacol., 6:87, 1957) showed that daily i.p. injections of saline or of the aldosterone analog, desoxycorticosterone acetate (DOCA), increased the voluntary intake of an otherwise rejected (15% v/v) ethanol solution. Interestingly, DOCA has been used in the treatment of Delerium Tremens (Smith, J.J., Quart. J. Stud. Alc., 11:190, 1950) and it has been noted (ibid) that the Addisonian crisis is similar biochemically and clinically to Delirium Tremens. In keeping with this theme, the present experiment investigated the effect of sodium hunger on the voluntary intake of ethanol.

Four different groups of female Wistar rats maintained on a nutritionally adequate (basal) diet were offered a choice between distilled water and either 3%, 6%, 12% v/v ethanol solutions or distilled water every third day (Phase I). Only ethanol (3 groups) or distilled water (1 group) was available on intervening days. When consumption had stabilized the animals were first switched to a low sodium (L.S.) diet (Phase II) and then to an L.S. diet in combination with a brief regimen (4 injections) of the diuretic furosemide (60 mg/kg). In the final phase the animals were returned to the basal diet. Control groups were treated identically except that they were maintained on the basal diet throughout.

For those animals which preferred ethanol while on the basal diet (3% and 6% groups), the L.S. diet in combination with the diuretic, but not the L.S. diet alone, produced a decrease in the preference for ethanol, a decrease in the absolute amount of ethanol consumed and a concomitant increase in water intake. Reintroduction of the basal diet in the final phase lead to a recovery of the ethanol consumption. None of the control groups showed similar changes.

These findings indicate that sodium hunger is a condition incompatible with the ingestion of appreciable quantities of ethanol. To what extent this effect is directly related to the electrolyte imbalance or secondary to a change in metabolic state, taste sensitivity or some other factor or combination of factors, remains to be explored.

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163.2 EFFECT OF AMPHETAMINE ON ETHANOL DRINKING IN RATS. P.M. Duncan. Psychology Dept., Old Dominion Univ., Norfolk, VA 23508.

The effect of chronic amphetamine (amph) treatment on rats' voluntary drinking of ethanol solution (ES) has apparently not been reported previously. Exp 1 examined this effect at 2 doses of d-amph (.8 and 4.0 mg/kg) in 18 male Long-Evans rats which had access to both H_2O and 5% ES during an 8-day baseline (BL) period. Three groups (n=6) each then received 8 daily injections of saline, low or high amph doses. Mean ethanol preference relative to H_2O (EP) in the high-dose grp dropped progressively on inj days 1-8 from a .78 BL to .30 on day 8. Low-dose grp mean EP gradually decreased from a BL of .72 to .31 on day 8, with the most pronounced decline occurring on days 5-8. Saline grp mean EP remained stable near its .88 BL. ANOVA revealed sig. diff. ($p < .05$) between each drug grp and the saline grp, but not between the 2 drug grps. In Exp 2, the effect of 10 daily amph inj (1.5 mg/kg) on subsequent EP in 8 rats not allowed concurrent access to (but previously familiarized with) ES was examined. Three other grps (n=8 each) included one given saline inj and no ES access for 10 days, and 2 grps given daily saline or amph inj concurrent with continued (post BL) access to H_2O and ES. All 4 grps had access to both ES and H_2O on days 11-16 during which daily amph or saline treatments continued as on days 1-10. A drop in EP, first seen on day 5 and persisting near a mean of .25 through day 16 (sig. diff., $p < .05$), emerged only in the grp receiving both amph inj and continued ES access on days 1-10. Mean EP for the saline-constant access grp remained near the BL level of .60. When the "delayed ES access" grps were again given ES-H_2O choice on day 11, both amph and saline-inj grps maintained high (at least .50), similar mean EPs on days 11-16. Since the decline in EP produced by daily amph injection emerged only (or became greater) after several days off such treatment, and did not occur at all if rats were familiarized with amph effects while not drinking ES, the results are consistent with the interpretation that ES intake potentiates and/or prolongs aversive amph effects and thus produces a conditioned taste aversion (CTA) for ES which eventually causes the decreased EP. Potentiation of amph effects by ethanol (injected IP) has been observed for amph-stereotyped behavior (Todzy, et al., Psychopharm 59: 143, 1978) and for amph-produced CTA to flavored liquid in a conventional CTA paradigm (Duncan, EPA convention, 1982). Pre-exposure to amph has been shown to reduce its effectiveness as a US for production of CTA (Cappell, et al., Psychopharm 43: 157, 1975). Although post-ingestional potentiation of amph effects by orally consumed ethanol has not been directly demonstrated, potentiation could logically occur and result in the decreased ES intake seen here.

163.3 EFFECTS OF ETHANOL 4 G/KG BODY WEIGHT IN RATS OF DIFFERENT AGES. E. M. Burns, T. W. Kruckeberg* and P. K. Gaetano*. Dept. of Nurs. Science, University of Illinois Medical Center, Chicago, IL 60612

The purpose of this research was to determine whether or not ethanol 4 g/kg body weight daily throughout the brain growth spurt would affect body temperature and if so, therefore confound neurochemical and/or morphological findings. Sprague-Dawley timed-gestation rats were obtained from the Holtzman Company (Madison, WI). On the day after birth, animals were weighed, pooled, and 8 pups randomly assigned per dam. Subsequently, animals were given ethanol 4 g/kg body weight (in two 2 g/kg doses three hours apart) daily from day 27 through 37 postconception (approximately day 6-16 postnatally). Age-matched isocaloric milk "pair-fed" controls and "handled controls (handled in the same manner as treated pups, including intubation without the administration of any substance) were included in this study. Ethanol solution (20% W/V, prepared from 95% alcohol USP) or the isocaloric equivalent of milk was administered via a metal intragastric tube.

Mean blood ethanol concentrations (BEC) at the peak level (45 minutes after the second 2 g/kg dose) was found to be 264.90 ± 68.27 mg% (N = 71). Body temperature was measured using a mini thermistor probe and telethermometer (Yellow Springs) prior to treatment or handling, at 45, 105 and 180 minutes after the first 2 g dose of ethanol, and at 60 and 180 minutes after the second 2 g dose of ethanol on days 27, 30, 34, and 37 postconception. Body temperature in ethanol-treated animals did not differ from that in the two groups of controls, which did not differ from each other.

Body temperature was measured similarly in additional animals on day 43 postconception (the day after weaning). In these animals, body temperature was significantly lower in the ethanol-treated as compared with controls at one time period only (105 minutes after the first 2 g dose). In ethanol-treated adult rats body temperature was significantly lower from 105 minutes after the first 2 g/kg dose throughout the remainder of the observation period (405 minutes) as compared with body temperature prior to ethanol treatment.

Additional controls were used to investigate the temporal pattern of the ontogeny of thermoregulation in the Sprague-Dawley rat. By day 41 postconception thermoregulation in developing rats approached that of adults.

Since no effect of ethanol 4 g/kg body weight occurred on any day throughout the brain growth spurt on body temperature, changes observed in synaptic neurochemistry and morphology are not due to the effect of ethanol on body temperature.

163.4 MEMORY FACILITATION BY POST-TRAINING ETHANOL: STRAIN DIFFERENCES. R.L. Alkana, D.A. Finn* and R.D. Malcolm*. Institute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

In contrast to the typical impairment induced by pre-training ethanol administration, recent studies demonstrate that immediate post-training injection of ethanol (0.75 - 4.5 g/kg) facilitates retention performance on a single trial passive avoidance task in C57 and Swiss Webster mice. The present study extended this investigation to a third mouse strain with a documented difference in central nervous system sensitivity to ethanol. Age-matched, drug-naive, male C57 BL/6J, Swiss Webster (Sim) and BALB/cJ mice were housed 4-5 per cage on a 12 hour light-dark cycle (0700 on) for two weeks before experimentation. After 4 days of pre-handling and saline injections (0.1 ml) to reduce stress, the mice were trained between 1500 and 1830 hours on a one-trial passive avoidance task according to a counterbalanced design using footshocks of 0.10 mA (Swiss Webster), 0.15 mA (C57) and 0.20 mA (BALB). Footshock levels were selected on the basis of pilot studies to produce approximately equivalent retention times in saline treated controls. Immediately after training, mice were injected i.p. with 3.0 g/kg ethanol (16% w/v) or an equivalent volume of normal saline. Retention was tested in the non-ethanol state one week after training. Retention was operationally defined as the difference between test session and training session latencies to leave the lighted start box and reach a point 8 cm into the darkened end of the two-compartment alley. Immediate post-training injection of ethanol significantly increased retention latencies in C57 and Swiss Webster mice compared to their respective saline injected controls ($p < 0.05$, Mann Whitney U), but did not significantly alter retention latencies in BALB mice. These strain differences in response to ethanol could reflect underlying differences in brain sensitivity to ethanol, hormonal or neurotransmitter responses or other factors which may prove useful in understanding ethanol's effects on learning and memory (Supported by NIAAA, ADAMHA Research Grant AA-04600).

163.5 EFFECT OF ETHANOL ON SPONTANEOUS MOTOR ACTIVITY (SMA) IN ALCOHOL-PREFERRING AND -NONPREFERRING RATS. Marshall B. Waller, William J. McBride, Lawrence Lumeng* and Ting-Kai Li*, VA Medical Center, Regenstrief Institute and Depts. of Psych., Biochem. and Medicine, Indiana Univ. School of Medicine, Indianapolis, IN 46223.

Low doses of ethanol (EtOH) reportedly are excitatory and potentiate behaviors such as SMA in several strains of mice and rats. To examine whether behavioral arousal is a correlate of EtOH preference the effects of low doses of EtOH on SMA were studied in the selectively bred preferring (P) and -nonpreferring (NP) lines of rats and in the Maudsley Reactive (MR/N) strain for which behavioral arousal has been reported (Kochar and Erickson, Alcoholism 6: 147, 1982). Alcohol naive male and female animals were given food and water ad libitum except during the test sessions. Food was removed 24 hr. before testing. After the intraperitoneal injection of saline or EtOH (0.0625 to 1.5 g EtOH/kg), SMA was monitored every 3 minutes for 30 minutes post-injection in an electronic activity monitor. Each EtOH dose was compared to saline (the paired t-test) for evaluation of statistical differences in activity. Female P rats showed an increase in SMA after the injection of 0.0625 and 0.125 g EtOH/kg while doses of 0.0625 to 0.5 g EtOH/kg increased SMA in P males. However, MR/N females exhibited increased SMA only at the 0.25 g EtOH/kg dose and MR/N males were more active after doses of 0.125 and 0.25 g EtOH/kg. In all cases, the maximum change in activity occurred within 9 min. post-injection. In sharp contrast, doses of 0.0625 to 0.5 g EtOH/kg failed to induce stimulation of SMA in either male or female NP rats. Doses of 1.0 or 1.5 g EtOH/kg consistently depressed SMA in all three strains of rats. Six minutes after the intraperitoneal injection of P female and MR/N male rats with 0.125 g EtOH/kg, cerebral blood EtOH concentrations were 15 and 12 mg%, respectively. Brain EtOH content was 21 and 16 mg/g, respectively. The intraperitoneal administration of 1.5 g EtOH/kg into P and MR/N animals elevated cerebral blood EtOH concentrations to 223 and 214 mg%, respectively and brain EtOH content was 286 and 269 mg/g, respectively 6 min. post-injection. These data suggest that P rats are more sensitive to stimulation by low doses of EtOH than either the MR/N or NP lines and that the CNS pharmacologic effects of low doses of EtOH may be an important variable in governing EtOH drinking behavior. The drinking scores, determined by free-choice drinking of 10% EtOH vs. water after SMA testing, for the P, MR/N and NP animals in this study were 6.6 ± .2, 3.3 ± .5 and 0.9 ± .3 g EtOH/kg/day. Therefore, the evidence is consistent with a positive relationship between EtOH preference and EtOH-induced locomotor stimulation. (Supported by Grants AA-03243 and MH-00203).

163.6 THE RELATIONSHIP BETWEEN CENTRAL AND PERIPHERAL ACETALDEHYDE OXIDIZING CAPACITY AND VOLUNTARY ETHANOL CONSUMPTION IN THE RAT. S. M. Socaransky*, C. M. G. Aragon*, F. Rogan*, Z. Amit, and Z. W. Brown. Center for Research on Drug Dependence, Dept. of Psychology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

The relationship between voluntary ethanol consumption and cerebral and hepatic aldehyde oxidizing capacity was investigated in the male Wistar rat. A significant correlation was observed between voluntary ethanol intake and levels of brain aldehyde dehydrogenase (ALDH) activity. Within the same animals there was no correlation between levels of hepatic ALDH activity and voluntary ethanol consumption. Levels of cerebral ALDH activity did not differ between ethanol exposed and control animals. Higher levels of hepatic ALDH activity were observed in ethanol exposed than in controls animals indicating possible induction or protection of the enzyme in the liver in response to ethanol. As a significant correlation existed between voluntary ethanol consumption and cerebral ALDH activity levels it is argued that brain ALDH activity may be integrated into the regulation of voluntary ethanol consumption. Preliminary investigations of cerebral ethanol oxidizing capacity and its relation to voluntary ethanol consumption also indicate a possible relationship between brain catalase activity and voluntary ethanol intake.

163.7 SUBCELLULAR DISTRIBUTION OF 3H-LEUCINE IN BRAINS OF ETHANOL TOLERANT VS. NONTOLERANT RATS AFTER PERIPHERAL ADMINISTRATION. D.D. Walczak* and H. Kalant* (SPON: P. A. Stewart). Dept. Pharmacol., University of Toronto, Toronto, Ontario M5S 1A8.

Rats were placed on a chronic treatment regimen known to produce tolerance to ethanol. Tolerance was assessed by a method developed in our laboratory in which rats were trained to cross a suspended dowel to obtain a food reward. Ethanol produced dose-dependent decrements in ability to obtain the reward, and tolerance developed to this effect. Duration of exposure was limited to six weeks to limit the development of chronic toxicity and metabolic tolerance. Ethanol or isocaloric sucrose was administered by daily gavage beginning at 3.0 g/kg/day. Doses were increased by 0.5 g/kg/day twice a week until 5.0 g/kg/day was reached. Doses were increased by 0.5 g/kg/day at weekly intervals thereafter until the level of 6.0 g/kg/day was reached. Tolerance development was measured at two-week intervals by constructing log dose-response curves using the selected behavioral test. Effects of 6 and 12 weeks treatment on the pharmacokinetics of ethanol and 3H-leucine were determined. After six weeks of treatment the animals were assigned to one of four experimental groups: Ethanol tolerant receiving ethanol (ET+E), ethanol tolerant receiving sucrose (ET+S), Sucrose controls receiving ethanol (SC+E), and Sucrose control receiving sucrose (SC+S). All animals were fitted with indwelling jugular cannulas 24 hr prior to receiving a test dose of either ethanol 3.5 g/kg or isocaloric sucrose, followed 30 min later by three i.v. injections of 3H-leucine, 0.5 mCi, spaced 30 min apart. Sixty min after the last i.v. injection the animals were sacrificed by decapitation and then subjected to subcellular fractionation according to the five-fraction scheme of deDuve et al. (Biochem. J. 60: 604-617, 1955). All fractions were analyzed for protein content and radioisotope concentrations. Groups ET E and ET S showed slight decreases in the protein content of whole homogenate and light mitochondrial fraction, and increased protein content in the 100 x g pellet and supernatant. Both ET groups showed increased incorporation of 3H-leucine into the heavy and light mitochondrial fractions, with the increases being significant in the light mitochondrial fractions. Ethanol withdrawn (WT S) subjects showed the highest degree of incorporation in these fractions. The fractions showing these changes have been subjected to electrophoresis on SDS-polyacrylamide gels to determine the specificity of labeling of individual protein bands. This data suggests that an increased turnover of light mitochondrial components is occurring in the ethanol-tolerant animals that is not observed in either sucrose control group.

This research was supported by the Alcohol and Drug Addiction Research Foundation of Ontario.

163.8 TEMPORAL CHANGES IN PLASMA LEVELS AND METABOLISM OF KETONE BODIES BY LIVER AND BRAIN OF C57BL/6J MICE AFTER ETHANOL AND/OR STARVATION. R. A. Schreiber and Y.-Y. Yeh*. Dept. of Biochem., UTCHS, Memphis, TN 38163 and Dept. of Nutrition and Metabolism, The St. Jude Children's Research Hosp., Memphis, TN 38101.

Two experiments were performed, on the hypothesis that the liver may metabolize ethanol (ETOH) to β-hydroxybutyrate (BOHB) and acetoacetate (AcAc), which may then be utilized by the brain. In Experiment I, levels of plasma BOHB and AcAc were determined for up to 31 hr. after either an injection of 5 g/kg of 20% ETOH, or after starvation, or both. Untreated control levels were .322 mM BOHB and .049 mM AcAc, ± .054 and .010 (SEM), respectively. No sex differences were found. Starvation elevated BOHB levels starting at 7 hr., and were significantly elevated (.922 mM) by 10 hrs. BOHB levels peaked (2.5 mM) at about 24 hr., then appeared to decrease to 1.8 mM at 31 hr. The AcAc/BOHB ratio began to fall from .225 ± .074, attaining a nadir of .046 at 15 hr.; due to the rise in BOHB levels. ETOH enhanced BOHB levels which peaked at 10 hr. (1.42 mM) and returned to control levels by 15 hr., while ETOH plus starvation caused an increase to 1.22 mM BOHB by 7 hr., and to 1.88 mM by 31 hr. The AcAc/BOHB ratio had fallen to .108 by 7 hr. and .058 by 10 hr., where it remained, approximately, to 31 hr.

Other mice were then given 5 g/kg ETOH ± starvation (Gps. E, or E + S) and were sacrificed for metabolic studies at times of ETOH-elevated ketone bodies (10 hr.), or starvation-elevated ketone bodies (24 hr.). Liver homogenates were incubated with 1-^{14}C palmitate; hepatic capacities for incorporation into CO_2 and ketone bodies were determined. Brain homogenates were incubated with 3-^{14}C AcAc; incorporation into CO_2 and fatty acids was determined. In controls, male livers utilize more palmitate for energy (423.6 vs 120.9 nmol/g/30 min), and female brains incorporate more AcAc than males (171.8 vs 358.7 nmol/g/2 hr.). There were no sex differences among experimental groups. Neither E nor E + S affected the capacity for CO_2 production from palmitate in liver or from AcAc in brain at 10 hr. At 24 hr., starvation led to an elevated capacity for liver utilization of palmitate. The capacity for AcAc synthesis was increased 70% at 10 hr. in both Gps. At 24 hr., the synthetic capacity was doubled in Gp. E + S, and had returned to control levels in Gp. E. In the brain, the capacity for fatty acid synthesis was 90% higher in Gp. E + S, and 70% higher in Gp. E at 10 hr; both had returned to control levels by 24 hr.

Taken together, the two experiments show that ETOH increases ketone body levels by accelerating hepatic ketogenesis. Since plasma BOHB levels determine brain utilization rates (Hawkins et al., Biochem. J., 122:13, 1971), ETOH could provide a potential source of ketone bodies for brain synthesis of lipids and for energy.

163.9 ALCOHOL AND THE AUDITORY BRAINSTEM RESPONSE: DOSE EFFECTS. Julia A. Lee,* Robert F. Berman, Donald W. Nielsen, and Eugene P. Schoener. Psychology Dept., Wayne State University, Otological Research Laboratories, Henry Ford Hospital, and Pharmacology Dept., Wayne State University School of Medicine, Detroit, MI 48202.

Ethyl alcohol is one of the few pharmacological agents to alter the auditory brainstem response (ABR), a series of 6-7 waves within the first 10 msec following an auditory stimulus. Chu, Squires, and Starr (Arch. Neurol.,35:596, 1978) demonstrated that an acute dose of alcohol (2-3 g/kg) produced slowing of the ABR waves. They administered only one dose of alcohol and measured the ABR at a single time point (45-60 min) following alcohol. The present study was undertaken to confirm and extend their results by administering a range of alcohol doses and recording the ABR over a longer time period.

Four adult male Long Evans rats were surgically prepared according to a recently developed procedure for ABR recording in unanesthetized, unrestrained rats (Lee, Abbott, Berman, and Nielsen, Neurosci. Abstr., 7:51, 1981). With this procedure the speaker-ear distance is held constant by attaching a miniature speaker to the rat's skull. Ethanol doses of 0.5, 2.5, and 5.0 g/kg, po were administered at intervals of at least 7 days. Sessions began with a 30-60 min baseline period followed by drug administration. ABR recording was continued for 2-3 hrs.

Slowing of the ABR waves with a moderate (2.5 g/kg) and a high (5.0 g/kg) alcohol dose was observed for all subjects. With the higher dose, wave slowing typically started sooner, lasted longer, and was more pronounced. A most interesting result was noted with the moderate alcohol dose. For two subjects, there was a biphasic effect characterized by an initial decrease in wave latencies followed by an increase (wave slowing). The wave slowing started 45 min after alcohol. With the 0.5 g/kg alcohol dose an early excitatory effect was seen for one subject. No other definitive ABR latency changes were noted at this lowest dose. These findings indicate that the ABR is sensitive to excitatory as well as depressive effects of acute alcohol doses. The excitatory effects observed would appear to correspond to the rising phase of blood alcohol levels.

Jones, Stockard, and Weidner (Electroenceph. clin. Neurophysiol., 49:23, 1980) found that when temperature was held constant, ABR wave slowing in response to acute alcohol did not occur. They suggested that alcohol's action upon the ABR reflects an indirect effect of alcohol-induced temperature change. Therefore, the relation of brain temperature to the present dose-dependent effects is now being investigated.

163.10 A GENETIC ANALYSIS OF ETHANOL RESPONSIVITY IN MICE. B.C. Dudek, M.E. Abbott* and T.J. Phillips*. Dept. Psychology, SUNY-Albany, Albany, NY 12222.

The selective breeding program of McClearn and Kakihana (Behav. Genet. 3:409, 1973) produced two lines of mice which dramatically differ in response to sedative-hypnotic properties of ethanol (ETOH). In our experiments, LS mice lose the righting reflex for over 100 min in response to a 3.8 g/kg dose of ETOH while the SS mice are similarly impaired for less than 30 min. A mendelian genetic analysis of this selected phenotype demonstrated an additive genetic system with no maternal effects, minimal interlocus interaction and no dominance. Biometrical genetic analysis computed the heritability of the character at.19 and estimated the number of genes controlling the character to be at least 13. The correlation between genotype means of the loss of righting reflex duration and blood ETOH levels at righting was .98, domonstrating that the genetic effect is almost completely due to tissue (neural) sensitivity to the drug and not dispositional factors. Male mice were somewhat more sensitive than females but this difference was small relative to the genetic effect. The depressant drugs tertiary-butanol and acetaldehyde produced longer loss of righting reflex in LS than in SS mice. Intermediate inheritance was present in the t-butanol experiment, but dominance to the LS genotype was found for acetaldehyde. Pentobarbital produced longer loss of righting reflex in SS mice, thus demonstrating the specificity of the selection program for alcohols and alcohol metabolites rather than sedative -hypnotics generally.

The stimulant properties of low ETOH and t-butanol doses were assessed with a measurement of their effects on locomotor activity. Both alcohols produced considerable activation in SS mice but very little in LS mice. F1 and F2 mice showed sub-hypnotic ETOH dose response curves intermediate to the two parentals. It is likely that some or all of the 13 or more genes differentiated by the selective breeding for the hypnotic dose sensitivity phenotype also predispose the differential stimulant properties of sub-hypnotic doses in the two lines. The number of loci involved in the difference between the two lines suggests that the ongoing work into the neural mechanisms underlying the ETOH sensitivity phenotype should find several biochemical and perhaps anatomical systems to be responsible.

Supported by the Research Foundation of the State University of New York

163.11 DOPAMINERGIC INTERACTIONS OF ETHANOL WITH NEUROTENSIN, β-ENDORPHIN AND BOMBESIN, G. D. Frye, E. Widerlov, R. A. Mueller, and G. R. Breese. Center for Alcohol Studies, University of North Carolina, Chapel Hill, N.C. 27514

Neurotensin (NT), bombesin (BOM) and β-endorphin (β-END) are neurobiologically active peptides which have been identified endogenously in many areas of the mammalian brain. Changes in behavior, neurochemistry and neurophysiology following their central administration suggests that these peptides and others may represent a new class of neuronal transmitter or modulator. Recently it has been shown that the central administration of NT, β-END and BOM can alter the actions of CNS active drugs. For example, we have found that ethanol-induced impairment of the aerial righting in mice is potentiated by intracisternal (IC) injection of these peptides (Frye, et al. Peptides 2, Suppl.1, 99, 1981). The mechanism of this interaction is not clear, but could involve brain dopamine (DA) systems. Ethanol has been widely reported to alter DA metabolism in brain and recently, NT, β-END and BOM also have been shown to act on this transmitter. Ethanol (2.25 g/kg i.p.) as a 10% (w/v) solution or saline was given 15 minutes prior to IC injection of NT (60 µg), BOM (45 µg), β-END (20 µg) or saline (25 µl) to unanesthetized rats. The animals were sacrificed 45 min after the IC injection. The concentrations of DA, DOPAC, and HVA, were determined in striata, olfactory tubercles and nuclei accumbens using high performance liquid chromatography. Ethanol alone consistently did not change DA, DOPAC or HVA concentrations in any of the three brain areas studies. In general, NT, β-END and BOM increased DOPAC concentrations in all brain areas except in the tubercles where NT had no effect and the stratum where BOM caused a decrease. HVA concentrations were also increased in the stratum and tubercles by NT and β-END, but only in the tubercles by BOM. Ethanol failed to alter changes in DA metabolites caused by the peptides alone. These results do not support the hypothesis that the potentiation of ethanol-induced depression by NT, BOM, β-END is due to a synergistic effect on DA mechanisms. (Supported by USPHS AA-02334 and HO-03110).

163.12 INTERACTIVE EFFECTS OF ANDROGEN AND ALCOHOL ON AGGRESSION IN MICE. Joseph F. DeBold and Klaus A. Miczek. Department of Psychology, Tufts University, Medford, MA 02155.

Both alcohol and androgen may substanitally modulate aggressive behavior in male mice and other species. In addition, alcohol administration alters androgen secretion through direct actions on the testes and by affecting gonadotropin secretion. It has been suggested that at least some of the effects of alcohol on aggression are mediated by the effects of alcohol on androgen levels. If this hypothesis is correct, then animals with very low androgen levels or artificially controlled androgen levels should show much less alteration in aggressive behavior after alcohol. We compared the behavioral response to alcohol in male and female mice and in castrated mice implanted with constant release capsules of testosterone.

Male and female CFW mice were housed in pairs. Within a few weeks these resident mice will repeatedly attack an unfamiliar intruder mouse placed into their home cage. In the first experiment two separate groups of resident male (n=20) mice were given 0, 0.1, 0.3, 1.0, 1.7 or 3.0 g/kg of ethanol p.o. Fifteen minutes later their attack and threat behavior toward a same-sex intruder mouse was measured. These doses had little effect on the time spent walking and rearing by the male residents but the 3.0 g/kg dose of alcohol significantly suppressed aggression. However, in females 0.1g/kg significantly enhanced aggression although 3.0g/kg reduced attack and locomotion. Male (n=11) and female (n=7) mice were also tested for the response to 3.0g/kg ethanol 5, 15, 30, 60 or 120 minutes after administration. In this time course study suppression of aggression lasted through the 120 min test in females, while males were beginning to recover to normal attack rates by 120 min. Another group of male mice (n=15) were castrated and implanted with 7.5mm silastic capsules of testosterone, which released constant high levels of androgen. These animals when given the same doses of alcohol as the intact males showed a shifted dose response curve. Doses of 1.0 and 1.7g/kg significantly increased aggression; 5.6g/kg ethanol was required to inhibit aggression. Blood alcohol levels were somewhat lower in males than females after the higher doses of alcohol but this difference was small and did not appear to be related to the biphasic action on aggression.

The results from these experiments demonstrate that the effects of alcohol on aggressive behavior are different in mice with high, normal and low levels of androgen. However, it does not appear that the effect of alcohol on aggression requires changes in androgen levels or that normal male levels of testosterone are necessary for this response to alcohol.

163.13 IMPAIRMENT OF CALCIUM-MEDIATED INHIBITION WITH CHRONIC ETHANOL TREATMENT MEASURED INTRACELLULARLY. D. Durand and P.L. Carlen. Depts. of Biomedical Engineering & Phsyiology, University of Toronto, Addiction Research Foundation and Playfair Neuroscience Unit, Toronto, Canada.

Chronic ingestion of ethanol has been shown to produce psychological, behavioral and morphological damage in animal models of ethanol induced brain damage. Although specific areas like the hippocampus have for example shown to be particularly affected very few studies have been aimed at the study of pathophysiology of this organic brain syndrome. Recently, however, extracellular in-vivo recordings in the hippocampus of animals fed ethanol in liquid diets for 5 months revealed significant impairment of inhibitory mechanisms. These results were however confounded by the presence of anesthetics. We have been able to confirm the depression of inhibition from chronic ethanol treatment by both extracellular and intracellular recording in-vitro and have studied the possible mechanisms.

For 5 months, male Sprague Dawley rats were provided ad-libitum but measured access to a liquid diet containing 35% of its calories as ethanol while a control group received isocaloric amounts of the same diet with ethanol replaced by maltrox-dextrins. One set of animals was withdrawn for 2 months and an in-vitro extracellular study was performed while another set was withdrawn for 3 weeks and intracellular recordings were obtained in-vitro.

Inhibition was measured extracellularly in the CA1 cell layer using an antidromic conditioning pulse followed by an orthodromic test pulse (stratum radiatum) at various delays. The inhibition in the ethanol fed animal was significantly decreased at a delay of 20 ms ($p<.05$ 2 tailed t-test). Intracellular recordings from 125 granule cells revealed that inhibitory post-synaptic potentials (IPSPs) were significantly reduced in ethanol-fed animals compared to their control while the resting potential, action potential, EPSP and rheobase currents were not affected. The after-hyperpolarization (AHP) following spike trains from depolarizing current pulses were also significantly reduced in ethanol animals. Identical results were obtained in a total of 24 CA1 cells from the same animal group. Therefore the two main inhibitory potentials (IPSP and AHP) are both depressed confirming the extracellular results.

Recent evidence points to a calcium-mediated potassium conductance as responsible for both the AHP and the late phase of the IPSP. This data therefore suggests that calcium mechanisms may be impaired by chronic ethanol treatment.

Work supported by NIH Grant R01 NS16660-02 and the Addiction Research Foundation.

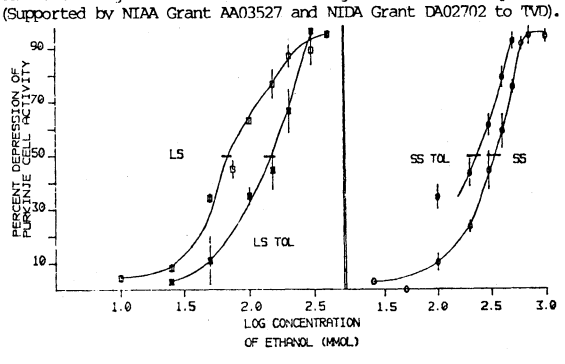
163.14 PHYSIOLOGICAL MEASURES OF ETHANOL WITHDRAWAL IN LONG-SLEEP AND SHORT-SLEEP MICE. D. M. Gilliam and A. C. Collins*. Inst. for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309.

In order to assess dependence liability in the long-sleep (LS) and short-sleep (SS) mice, selectively bred for a difference in acute ethanol sensitivity, different groups of mice were administered an ethanol or a pair-fed control diet for either 7 or 14 days. Physiological measures of withdrawal were then obtained during a 24-hr period that began immediately after termination of treatment by noting changes in rectal temperature (T), pulmonary ventilation (PV), heart rate (HR), and acoustic startle response (ASR). Withdrawal effects observed following the 7-day ethanol treatment were less severe and occurred more quickly after treatment terminated than did those observed following the 14-day treatment period. SS mice consumed more ethanol (g/kg/day) than LS mice under both treatment conditions. Withdrawal severity following the 7-day treatment did not differ between the SS and LS mice; however, effects observed following the 14-day treatment were more severe in the SS than in the LS mice. Peak withdrawal was characterized by a general depression in T, PV, and HR, but the time course of the effect differed among measures. ASR was depressed throughout most of the withdrawal period.

Concurrent measurement of several physiological processes during ethanol withdrawal, rather than use of only a single measure, may better indicate variations in neural and systemic functioning.

163.15 ACUTE ETHANOL EFFECTS ON PURKINJE NEURON SPONTANEOUS ACTIVITY IN THE IN VITRO CEREBELLUM OF ETHANOL TOLERANT LONG AND SHORT SLEEP MICE. A.S. Basile* and T.V. Dunwiddie (SPON: D. Taylor). Dept. of Pharmacology, Univ. Colorado Health Sci. Center, Denver, Co. 80220.

Ethanol-induced changes in spontaneous activity of Purkinje neurons were compared in cerebellar slices from ethanol tolerant and naive LS and SS mice. The LS and SS mice are selectively bred to show high and low sensitivity to the soporific effects of ethanol. LS and SS mice were made behaviorally tolerant to ethanol with daily i.p. injections of 4.5 or 5.0 g/kg ethanol,respectively, for 5 consecutive days. On the 6th day, the mice were sacrificed and parasagittal slices were prepared from the cerebellar vermis. Slices were normally perfused with a modified Ringer's solution containing 1mM Ca^{2+}, 3.9 mM Mg^{2+}, 4.5 mM K^{+} and 0.001% H_2O_2, saturated with 95% O_2/5% CO_2. This medium eliminates almost all synaptic transmission in the slice. Extracellular recordings were made from spontaneously firing single Purkinje neurons. Ethanol was added to the perfusion medium in concentrations of 25-500 mM and drug-induced depressions in Purkinje cell spontaneous activity were quantified. The EC_{50} values for these depressions in firing rate were determined by Hill plots to be 75±8 mM and 327±29 mM in non-tolerant LS and SS mice, respectively. However, while repeated ethanol administration decreased the sensitivity of Purkinje cells in LS mice (EC_{50}=131±29 mM), the SS mice showed an increased sensitivity (EC_{50}=216±29 mM). These shifts in dose response curves between tolerant and non-tolerant mice are significantly different at the $p<0.05$ level, as indicated by the lack of overlap in the EC_{50} 95% confidence levels (horizontal bars, below). In conclusion, differential electrophysiological responsiveness to ethanol in tolerant LS and SS mouse lines is maintained in the in vitro cerebellum, and repeated ethanol treatments can induce significant shifts in Purkinje cell sensitivity to ethanol. (Supported by NIAA Grant AA03527 and NIDA Grant DA02702 to TVD).



163.16 FETAL EXPOSURE TO ETHANOL POTENTIATES OPIOID STRESS ANALGESIA IN ADULT RATS. L.R. Nelson, J.W. Lewis, J.C. Liebeskind, B.J. Branch*, and A.N. Taylor. Departments of Psychology and Anatomy and Lab. of Neuroendocrinology, Brain Research Institute, UCLA and Brentwood VA Medical Center, Los Angeles, CA 90024.

Taylor et al. (Pharmac. Biochem. & Behav., 1982) have demonstrated that rats exposed to ethanol in utero display an enhanced release of corticosterone as adults in response to certain stressors, such as cardiac puncture or noise and shake, but not others, such as fasting or cold stress. In the present study, we investigated a behavioral response, analgesia, to another type of stress, inescapable footshock. Lewis et al. (Science, 1980) described two forms of stress analgesia elicited by different parameters of footshock delivery; one form is opioid mediated, and the other appears not to be.

Subjects were female offspring of Sprague-Dawley dams fed either a 5% w/v ethanol-containing casein-supplemented liquid diet (BioServ) ad lib, pairfed an isocaloric liquid diet without ethanol, or given lab chow and water ad lib from gestation day 8 to birth. At birth, all pups were cross fostered to normal dams who had food and water available ad lib. At approximately 150 days of age, rats were tested for stress analgesia. Prior to footshock, baseline pain sensitivity was measured with the tail-flick test. Rats were then exposed to either prolonged/intermittent (2.5 mA, 1 sec on per 5 sec for 10 min) or brief/continuous (2.5 mA, for 2 min) footshock stress that produce opioid and non-opioid analgesia, respectively. After footshock termination, tail-flick latencies were recorded at 1 minute intervals for the first 9 minutes, and at 2 minute intervals from 9 through 15 minutes. All animals received both forms of footshock in a counterbalanced order separated by 1 week.

Rats prenatally exposed to ethanol (n=16), were significantly ($p < .05$) more analgesic after the prolonged/intermittent (opioid) footshock than offspring of pairfed (n=11) or normal (n=8) dams. Rats in all three groups showed similar analgesia following brief/continuous footshock.

These results are congruent with the results of Taylor et al. (1982), and add prolonged/intermittent inescapable footshock to the list of those stressors to which rats prenatally exposed to ethanol are more sensitive in adulthood. In addition, these results suggest that prenatal ethanol exposure produces long-term effects on the functioning of endogenous opioid-mediated analgesia systems. (Supported by Veteran's Administration Medical Research Service and NIH grant NS07628.)

163.17 DEGENERATIVE CHANGES OBSERVED IN THE SCIATIC NERVE OF RATS. AN EXPERIMENTAL MODEL OF ALCOHOLIC NEUROPATHY. J.P. Machado-Salas, G. Espinosa*, J. Espinosa* and P. García. Lab. Neuromorfología. UIICSE. ENEP Iztacala. UNAM. P.O. Box 314. Tlalnepantla 54030 Edo. de Mexico. MEXICO.

Even though the association of alcoholism and peripheral neuropathy in humans was established by Lettsom, two hundred years ago, it is until fairly recently that most of classical works have been done. Many unsolved facts are claiming for a more experimental approach. On this regard, the study of primary effects of ethanol on peripheral nerves, without involvement of dietary factors, deserves special mention.

We have raised, under similar environmental conditions, two groups of Wistar rats: control and experimental. The latter, were allowed to drink from a 20% (v/v) ethanol solution for up to 24 months. Both groups were fed with balanced rat food, ad libitum. Their daily in take of liquids was recorded every day, and their change in weight, every other day. In this regard, there was not a significative ponderal difference between groups. Also, there were not clinical signs of dietary defficiences. Smaller groups of 6 to 8 rats, were sacrificed every 4 months, and their sciatic nerves processed for light and electron microscopic studies. Hematoxilyn & Eosin, Guillery's, luxol fast-blue and Bodian's methods were used for the former.

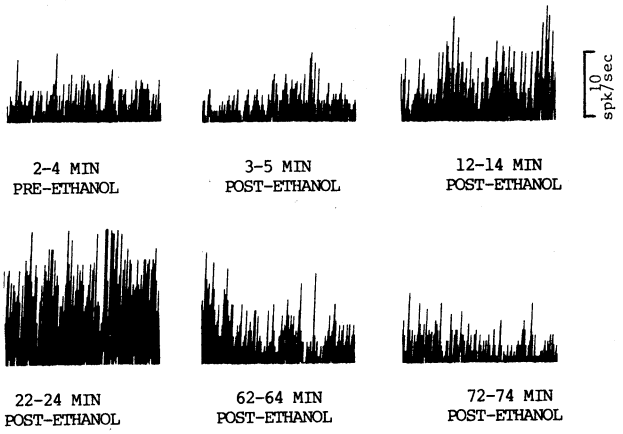
The sciatic nerves of long-term alcoholized rats showed: a) Irregular contours of the myelin sheaths and axones, b) Fragmentation of axones, c) vacuolization of myelin sheat and or axones, d) changes in diameter and trajectory of the same axon. On the other hand, the E/M showed: a)Round, patch-like areas in the myelin sheat, wich depicted abnormally separated interlameller spaces, b) Distorted, degenerated axones, loaded with dark bodies, fragmented tubules and degenerated mitochondria, c) Axoplasmic vacuoles filled with few, widely separated tubules and small, dark mitochondrion. The presence of abundant collagen was evident, as well as rather abundant fibres and mononuclear cells and capillaries among fibres.

These findings are very similar to those described in human alcoholic patients, therefore, we conclude that the chronic administration of ethanol, in well-fed rats, producessevere pathological changes in the peripherical nervous system. Therefore, we propose this model as an experimental paradigm of alcoholic neuropathy.

(Partial financial support was given by Instituto Mexicano de Psiquiatría)

163.PO ELECTROPHYSIOLOGICAL EFFECTS OF ACUTE ETHANOL ON OLIVO-CEREBELLAR NEUROTRANSMISSION. Joseph Rogers, G.R. Siggins, G. Aston-Jones, L.Y. Koda, and F.E. Bloom. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, La Jolla, CA 92037.

Ethanol, given in a single, acute IP dose under chloral hydrate anesthesia, increases climbing fiber-mediated bursts in cerebellar Purkinje cells. Climbing fibers originate in the inferior olivary nucleus (ION) of the brainstem. When spontaneous single unit, multiple unit, or field potential electrical activity is recorded in the ION before and after acute IP ethanol (2 g/kg), increased activity is consistently observed. A typical example is shown in the ratemeter records below. ION electrode placements were histologically (by lesion) and/or physiologically (by stimulation of contralateral hindpaw) verified in all cases.



Taken together with our cerebellar recordings, these data suggest that altered Purkinje unit firing after ethanol is secondary to activation of ION firing. Deranged firing of the ION may provide one of several mechanisms for the well-known, socially-catastrophic effects of ethanol on motor performance.

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164.1 GOLD THIOGLUCOSE LESION FORMATION: NEURAL SPECIFICITY.
Danley F. Brown* (SPON: D.D. Rigamonti). Div. of Combat Casualty Care, Letterman Army Inst. of Research, San Francisco, CA 94129

A single intraperitoneal injection of gold thioglucose (GTG) will cause a bilateral lesion in the ventromedial hypothalamus (VMH). This pathological phenomenon produces a sustained hyperphagia and obesity, presumably by destroying VMH glucosensitive neurons controlling feeding behavior. Recently, controversy has developed concerning the specificity of GTG-induced necrosis in the VMH. Since the hypothalamus does not possess a strong blood-brain barrier, it is postulated that GTG-induced VMH necrosis is brought about by a breakdown in the endothelium of the area microvasculature. The resulting ischemia leads to brain cell death and lesion formation. This idea of a nonspecific GTG-induced lesion in the hypothalamus is attractive, but inconsistent with other evidence. For instance, although other circumventricular organs, in addition to the VMH, apparently can be damaged by a GTG challenge, the median eminence is always spared. This observation is important, since the blood-brain barrier permeability of the median eminence is similar to that of other hypothalamic regions apparently affected by GTG. Recent work also suggests that linkages may be present among the circumventricular organs in the hypothalamus, thus accounting for extra-VMH damage. Using a small dose of GTG (100 mg/kg), Debons et al (J. Pathol. 129:73, 1979) have clearly shown morphologically that initial GTG damage in the VMH is perivascular. Dissolution of neural structures and edema were apparent adjacent to intact undamaged capillaries. In this study we chose to further investigate the initial process of GTG lesion formation in the VMH at the ultrastructural level in mice. Two doses of GTG were utilized, 300 and 800 mg/kg. Depending on the GTG dose, initial VMH destruction appeared between 4-6 hours after the GTG challenge. This damage was manifested by apparent extracellular edema and distention of the neuropil. Although GTG-induced necrosis appeared to commence adjacent to capillaries, occasionally the regional edema and dilated extracellular areas were not confined to the pericapillary space. By 12 hours, the animals treated with 800 mg/kg GTG exhibited extensive cellular destruction. Mice challenged with 300 mg/kg GTG showed similar results, but to a lesser degree. Complete dissolution of the cytoplasmic matrix was evident; nuclei were pycnotic, mitochondria were swollen, and synaptic figures were disrupted. Our results strongly support the idea that GTG lesion formation is a substrate-specific toxin for the neural cellular matrix in the VMH. The data also lend credence to the hypothesis of the existence of a glucoreceptor participating in feeding behavior.

164.2 DIFFERENCES IN CELL BODY CROSS-SECTIONAL AREAS OF NEURONS IN OBESE AND LEAN MICE. G.A.Oltmans and P.E. Meyers* (SPON: P.C. Tang). Departments of Pharmacology and Anatomy, The Chicago Medical School, N. Chicago, IL 60064.

In many respects the obesity syndrome of the obob mouse resembles that of the rat with lesions of the ventromedial hypothalamus (VMH). Consequently, it has been proposed that the syndrome in the obob mouse may in part result from a hypothalamic defect. Recently, Bereiter and Jeanrenaud (Br. Res. 165:249-260, 1979) reported decreased cell body sizes in several brain regions, including the VMH, in the obob mouse. In the current study we have repeated this work on VMH cell size in animals younger and older than those studied by Bereiter and Jeanrenaud in order to determine if this is a constant condition in the obob.

Frozen sections from 6 week and 12 month old obob mice and their age-matched lean littermate controls were cut at 25 μ and stained with thionin. The VMH was divided into 4 quadrants, dorsomedial (DM), ventromedial (VM), dorsolateral (DL), and ventrolateral (VL). Photomicrographs of these quadrants were taken from both left and right sides resulting in 8 positive transparencies per animal. Transparencies were projected onto poster paper and 25 cells/transparency were drawn for a total of 200 cells/animal. Only those cells with a focused nucleolus were analyzed. Total magnification was 4,000 x. Error in reproduction and measurement of cells from slide to paper was measured at less than 3%. Measurements were made with a polar planimeter.

In 6 week old obob mice the cell sizes were significantly smaller in the DM and VL aspects of the VMH nucleus when compared to lean controls. In contrast, at 12 months of age there were no significant differences between obob and lean mice in cell body sizes in any of the regions analyzed, and, if anything, there was a tendency for the cell size to be slightly larger in the obob mice. A comparison of the 6 week and 12 month old animals indicates that cell size remained about the same in obob mice at the different ages, but that cell size decreased in the older lean mice.

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164.3 FRAGMENTED INGESTIVE PATTERNS FOLLOWING SEPTAL LESIONS IN RATS
J. C. Mitchell, F. W. Flynn*, L. A. Evey* and T.L. Steele Psychology Dept. Kansas State University, Manhattan, KS 66506

In a first experiment, feeding on a balanced liquid diet and drinking patterns were measured in groups of rats with septal damage and in unoperated controls. Measurements were made with drinkometers for one hr following 24 hr deprivation periods and were recorded on a multichannel event recorder. Feeding measures included: total food and water intake, number and size of meals or feeding bouts, and number and duration of intrameal intervals. Septal rats did not differ from controls in total food or water intake. When a meal was defined as the consumption of at least .5 gm food separated by greater than 1.0 min septals were found to consume many small meals whereas controls generally ate one large meal. However, when a meal was defined as the consumption of at least .5 gm followed by an interval greater than 10 min, (a commonly used definition, e.g. LeMagnen & Davos, 1980) no differences in meal size were found, with both septals and control animals generally consuming one meal.

In order to account for these findings, this study was replicated using the same feeding and drinking measures and the animals behavior was monitored and recorded using a television camera. The incidence of grooming and exploratory behavior was also recorded on the event recorder showing feeding and drinking. The feeding and drinking patterns observed in this study were similar to those of the first study. The septal rats spent much greater amounts of time grooming and exploring the cage and slept rarely. Normals showed the typical satiety sequence of eating followed by grooming, and then by sleep. The eating of the septal rats was constantly interrupted by bouts of grooming, exploring or drinking with no orderly behavioral sequence. Thus, the septal animal appeared to exhibit the "fragmented behavior" described by Rowland (1977) in animals with lateral hypothalamic lesions. It is not yet clear as to whether it is more appropriate to describe animals with septal lesions as having fragmented behavioral patterns or as animals who eat small meals.

164.4 INGESTIVE RESPONSES TO HOMEOSTATIC CHALLENGES IN RATS WITH ABLATIONS OF ANTEROLATERAL NEOCORTEX. S.W. Kiefer, C.V. Grijalva, M.W. Gunion*, P.H. Cooper, and D. Novin. Dept. of Psychol. and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Ablations of the anterolateral neocortex (Braun, JCPP, 89:507, 1975) or prefrontal neocortex (Kolb & Nonneman, JCPP, 88:806, 1975) in rats induce a syndrome of feeding deficits and sequential stages of recovery similar, but shorter, than that after lateral hypothalamic (LH) lesions. In the present study, it was determined whether rats with anterolateral neocortex ablations would also show the particular deficits following homeostatic challenges that have been demonstrated in LH lesion rats.

Male Sprague-Dawley rats were placed into four groups: Group AC (n=19) was given anterolateral neocortex ablations; Group BWC (n=19) was a body weight control group; Group CC (n=15) was given control ablations of dorsal-posterior neocortex; Group N (n=20) was the normal control group. Following postoperative recovery the rats were tested for food and water consumption in a series of homeostatic challenges: glucoprivation induced by 2DG (125, 250, and 500 mg/kg) and insulin (4, 8, and 16 U/kg), food consumption in the absence of water and following food deprivation, water consumption in the absence of food and following water deprivation, and water consumption following ip hypertonic (5 ml of 1.0 M NaCl ip) saline injections. Both short term (1-3 h) and long term (3-24 h) consumption measures were taken.

Groups CC, BWC, and N showed similar dose response increases in food and water consumption following 2DG administration. Group AC increased water consumption in a dose-dependent manner but ate significantly less than controls with the 500 mg dose. With the insulin injections, all groups showed a significant increase in food and water consumption and there were no group differences. Likewise, there were no group differences in the amount of food consumed following periods of food deprivation (8, 12, or 24 h), the amount of water consumed following 24 hr water deprivation, or the amount of water consumed following hypertonic saline injections. Rats in Group AC were prandial drinkers because these rats consumed significantly less water in the absence of food (12 and 24 hr food deprivation periods).

The present results indicate that, on the whole, rats with anterolateral neocortex ablations respond normally to glucoprivic challenges although their response to high doses of 2DG may be impaired. Additionally, rats with ablations of anterolateral neocortex display prandial drinking, something characteristic of LH rats.

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164.5 DOES CORTICOFUGAL FIBER DAMAGE CONTRIBUTE TO THE LICKING AND EATING ABNORMALITIES OF DECORTICATE AND LATERAL HYPOTHALAMIC LESIONED RATS. I.Q. Whishaw and B. Kolb. Dept. of Psychology, Univ. of Lethbridge, Lethbridge, Alberta Canada, T1K 3M4.

Rats that receive bilateral lateral hypothalamic (LH) lesions are initially aphagic and adipsic but recover eating and drinking in well described stages. Decorticated (DC) rats are aphagic and adipsic but do not actively or passively reject food as do LH rats. Their recovery is characterized by an increase in motor efficiency and they typically require training before they spontaneously drink. Are the deficits in these animals related? We measured tongue extension during licking and cookie mash eating speed in DC and LH rats. DC rats were unable to extend their tongue more than 2 mm even after prolonged recovery, and were unable to eat 2 g of food in less than 60 sec. LH rats were initially as impaired, but over 30 days tongue extension improved to 75% of a normal 13 mm, and eating speed increased to 12 sec, vs 3 sec for control rats.

The deficits in tongue use and in eating speed were then demonstrated in a disconnection paradigm involving unilateral LH damage and contralateral neocortex removal. The abnormalities were as severe in these rats as was found in DC rats. This experiment suggested that a corticofugal system common to the cortex and LH could underlie the impairments. Varying the lesion location in the cortex and brainstem showed that the cortical source of the impairments was centered in the orbital frontal motor cortex with lesser contributions from surrounding neocortex. The brainstem source was located in the far lateral hypothalamus, from the level of the anterior ventromedial nucleus to the level of the substantia nigra. Corticofugal fibers involved in the control of head musculature have their origin in the lateral frontal cortex, and project in the cerebral peduncles adjacent to, or in, this area of the LH.

So yes, the correlation between effective lesion locations for producing tongue and eating abnormalities and the projection of corticofugal fibers is sufficiently close to suggest that these motor fibers are importantly involved in the aphagia and adipsia of LH and DC rats. Currently, studies are directed toward distinguishing corticofugal fiber damage-induced impairments from impairments that follow dopamine depletion and sensory system damage.

164.6 BODY WEIGHT REGULATORY CONSEQUENCES OF SUBCUTANEOUS INFUSION OF QUININE IN THE RAT. John P. Heybach* & Peter C. Boyle* (SPON: H. N. Sapru) Central Research Department, General Foods Corp., White Plains, NY 10625.

Adult male rats (S.D.; 345-365g) were allowed either ad libitum (AL; n = 8) access to powdered chow or were restricted to 60% (R; n = 8) of ad libitum intake for 7 days. On day 7 four rats from each group were implanted subcutaneously with Alzet minipumps containing quinine HCl (Q: 350µg/µl in absolute ethanol) while the remaining four rats in each group were implanted with pumps containing only vehicle (V). Vehicle or drug solutions were delivered at a rate of 2µl/hour. Following surgery, all rats were allowed ad libitum access to powdered chow for seven days. Food intake, corrected for spillage, and body weight were recorded daily. The R rats lost weight over the seven days preceding pump implantation. The AL-Q rats became hypophagic and lost weight during Q infusion with body weights stabilizing on days 14-15. Vehicle infusion had no effect. The R-Q rats became hyperphagic and gained weight during the Q infusion with body weight stabilizing on days 14-15 at the same level as that for the AL-Q rats. These results indicate that quinine's effect on body weight is not dependent upon its presence in food. Furthermore, quinine results in the attainment of an altered body weight maintenance level that is achieved by either decreases or increases in food intake depending upon the relationship of existing body weight to the new Q-induced level. We conclude that Q reduces the level around which body weight is actively regulated presumably by a pharmacologic mode of action.

164.7 MAINTENANCE AND DEFENSE OF ALTERED BODY WEIGHT LEVEL DURING CHRONIC INGESTION OF A QUININE ADULTERATED DIET. Peter C. Boyle* & John P. Heybach* (SPON: M. LEVITT) Central Research Department, General Foods Corp., White Plains, NY 10625.

In an attempt to assess the influence of chronic ingestion of quinine on body weight regulation, separate groups of adult male rats (S.D.; 350-370g) were allowed ad libitum access to either powdered chow (C) or chow adulterated with 0.4% (w/w) quinine sulfate (Q) for 28 days. On days 28-42 one group of rats from each dietary condition (n = 10/grp) was restricted to 60% of the daily food intake of its respective ad libitum control, followed on days 42-54 by unrestricted access to their respective diets. Food intake, corrected for spillage, and body weight was recorded daily throughout. Rats ingesting Q were initially hypophagic and lost weight. Food intake gradually recovered, but body weight remained at a reduced and constant percentage of C weight. During the restriction and refeeding periods both Q and C rats lost and subsequently regained equivalent amounts of weight. The Q and C rats showed a relative hyperphagia on the first refeeding day following food restriction, although this was more pronounced in the C group. From day 7 on the Q and C rats displayed similar changes in the gross efficiency of food utilization under ad libitum, restricted and refeeding conditions. These results indicate that following the acute phase (days 0-7) of hypophagia and weight loss, Q rats gain weight at the same rate and respond to a food restriction and refeeding manipulation in a fashion similar to C eating rats. We conclude that quinine ingestion does not compromise the animals' ability to respond to regulatory challenges but rather reduces the level at which body weight is regulated.

164.8 THE LATERAL HYPOTHALAMIC SYNDROME: IMPORTANCE OF THE ANSA LENTICULARIS IN AFFECTING THE ORIENTATION PHASE OF FEEDING BEHAVIOR. W. J. Kuenzel* (SPON: J. Barrett). Dept. of Poultry Sci., Univ. of Maryland, College Park, MD 20742.

The lateral hypothalamic syndrome, as originally proposed by Teitelbaum and Epstein (Psychol. Rev. 69:74, 1962) has been a useful model for documenting feeding and drinking deficits and stages of recovery following bilateral hypothalamic lesions. A controversy has arisen in the avian literature regarding the length of time required to recover from feeding deficits following bilateral lesions directed to various sites in the prosencephalon. The most extensive studies documented to date suggest that aphagia and deficits in grasping and mandibulation can persist for weeks following bilateral destruction of quinto-frontal structures in pigeons (Zeigler, H. P., Adv. Study Beh., Vol. 7:286, 1976). In contrast, bilateral lesions produced in several hypothalamic and thalamic sites effected transient aphagia in the chick (Kuenzel, W. J., Physiol. Behav. 28:237, 1982).

In an attempt to resolve the issue, feeding and drinking behavior of the adult fowl were first observed. A repeatable feeding sequence was recognized in all hens. It could be conveniently partitioned into 5 distinct phases which were later used to define a lateral hypothalamic syndrome for this species following several placements of bilateral lesions within the diencephalon. The 5 phases include (1) Arousal and food recognition, (2) Orientation (positioning of the body and head toward specific food particles), (3) Grasping (rapid opening and closing of the bill tip about a food particle), (4) Mandibulation (movement of feed particles from bill tip toward back of mouth), and, (5) Swallowing.

Several hens were lesioned throughout the diencephalon and mesencephalon. Food and water intake, feeding behavior and feeding efficiency were monitored before and after surgery. Similar to a previous study with chicks, adult hens showed transient aphagia (1-5 days) following lesions to the quinto-frontal tract and ansa lenticularis. However, the diet fed in both studies was mash. When a whole-grain diet such as wheat was alternated with the mash diet every 4-5 days, aphagia was scored up to 15 days with the whole grain diet compared to 0-3 days with the latter. Observing feeding behavior before and after surgery suggested that bilateral lesions to the ansa lenticularis resulted in orientation deficits. More persistent aphagia was produced on whole grain diets when two or more deficits in the feeding sequence were disrupted, particulary a combination of orientation and grasping deficits. The texture of the diet greatly affected the extent of aphagia. (Study conducted during tenure of a Fulbright-Hays Fellowship to Britain, Poultry Research Center, Scotland.)

164.9 GASTRIC AFFERENT INPUTS TO THE MESENCEPHALON AND HYPOTHALAMUS. I. Zarco de Coronado, F. C. Barone and M. J. Wayner. Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210

Male hooded rats were anesthesized with urethane and prepared for hypothalamic and mesencephalic single neuron recording. A gastric balloon or catheter was positioned in the stomach and the stomach was filled while simultaneously monitoring mesencephalic central gray (CG) or lateral hypothalamic (LH) neuronal activity. In order to evaluate pressure associated effects, balloons having different elastic properties were utilized for stomach distension. Those balloons requiring the highest pressures for filling (> 100 Hg) significantly changed the discharge frequency of LH and CG neurons when filled from 0-10 ml. Usually, lowering the temperature of water used to fill the gastric balloon increased the change in discharge frequency. Neural responses were eliminated by spinal cord transections but were unaffected by bilateral cutting of the cervical vagus nerves. The filling of more elastic balloons (50-80 mm Hg) in the stomach was not as effective in altering neural activity, and an increase in filling velocity (from 0.4 ml/sec up to 1 ml/sec) was required before changes in neural activity were observed. The responses observed during control experiments in which balloons were filled in the intraperitoneal cavity and the esophagus were not correlated with gastric distension responses. In other experiments infusions of solutions were made directly into the stomach, thus producing a more natural gastric distension ($\sim$ 30 mm Hg). Although even at higher filling velocities few central neurons were affected, distension responses observed under these conditions could be eliminated by bilateral transection of the vagus nerves. Also, the effects of gastric infusions using different solutions on neural activity were evaluated. When the effects of distension were controlled, some neurons appeared to be sensitive to osmotic changes in the stomach. In other experiments, animals were prepared for gastric stimulation and simultaneous recordings of electrocardiographic (EKG) and cortical electroencephalographic (EEG) activity and blood pressure were made. For the various balloon types or filling temperatures, gastric stimulation always resulted in a similar increase in blood pressure. No significant effects on EKG and EEG were observed. These results illustrate that both spinal and vagal afferents are important in the mediation of different types of gastric stimulation to LH and CG neurons. In addition, some neurons are sensitive to changes in gastric osmolality. These effects are probably important in mesencephalic and hypothalamic mechanisms controlling ingestion and autonomic nervous activity.

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164.10 EFFECTS OF BILATERAL NUCLEUS ACCUMBENS DAMAGE ON SCHEDULE INDUCED AND SCHEDULE DEPENDENT BEHAVIORS. D. L. Armstrong,* M. J. Wayner, F. C. Barone, B. B. Falk,* P. A. McGrattan* and S. M. Jones.* Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210.

Small electrolytic lesions in the nucleus accumbens produce deficits in schedule induced drinking. Male rats were reduced to 80% of ad lib feeding body weight and were trained to press a lever for 45 mg food pellets on a FI-1 min schedule. When schedule dependent lever pressing and schedule induced licking and drinking stabilized during 30 min daily test sessions animals were anesthesized with Equi-Thesin and prepared for surgery. Each animal received two bilateral lesions in the accumbens that damaged both the medial and lateral portions of the nucleus. Lesions were produced by passing 1 mA anodal current for 15 sec through insulated tungsten wire. Control animals received identical treatment except that no current was passed through the electrodes. Animals were also examined weekly for open-field activity and performance on sensorimotor tests. Following surgery the animals with lesions showed decreased lever pressing, licking, and water consumption for a 3 week period and then recovered to pre-surgery baseline levels of responding. Control animals were not affected. Home cage food and water consumption remained stable in both conditions. Results indicate that damage to the nucleus accumbens disrupts lever pressing and schedule induced drinking 3-4 weeks, then these behaviors appear to return to normal values. There were no observable effects on home cage ingestion. These results partially support the findings of Robbins and Koob (Nature 285: 409-419, 1980) who report that 6OHDA infusion into the accumbens-septi impairs development of schedule induced drinking.

(Supported by NINCDS USPHS grant No. 13543.)

164.11 CNS SITES OF NALOXONE INDUCED HYPODIPSIA: A MAPPING STUDY. D. A. Czech[1], E. A. Stein[2], M. J. Blake[2]* and O. O. Yang[2]*. Depts. of Psychology[1] and Biology[2], Marquette Univ., Milwaukee, WI 53233

Recent studies have indicated that narcotic antagonists can suppress appetitive behaviors in rodents. Both food and water intake is significantly reduced following systemic injections of either naloxone or naltrexone, with water intake generally agreed to be more sensitive. These observations have been interpreted to implicate an endogenous opiate system in some aspect of appetitive behavior - perhaps the reinforcing qualities of the stimuli. While this drinking effect has been shown to be quite robust and CNS mediation strongly indicated, relatively little is known about specific CNS mechanisms and/or sites of drug action. We report here an initial mapping study of CNS nuclei involved in naloxone suppression of drinking behavior in the rat.

Male, Holtzman rats were implanted with bilateral guide cannula aimed at the preoptic area, lateral hypothalamus, lateral ventricle or nucleus accumbens. Following a 1 week recovery period, rats were placed on a 23 hour water deprivation schedule with one hour water access given daily between 1100-1300 hours. On test days, 33 gauge injection needles were inserted into the guides and 0.5 μl of either naloxone or saline was injected bilaterally over 30 seconds. Rats were returned to the test cages immediately and allowed to drink water for 1 hour. No food was available during this test period. Water consumption was measured at 5, 10, 15, 30 and 60 minutes post injection. Naloxone doses employed were 12.5, 25 and 50 μg. Each rat was tested at each dose (plus saline) in an incomplete Latin Square design with test days separated by 3-4 days. All loci were confirmed histologically.

ANOVAs were performed with significance level set at $p<.05$. At 15 minutes post injection, fluid intake for the 50 μg dose was significantly less in the POA and LH groups when compared to corpus callosum and cortex control sites. However, at 30 minutes, the NAS as well as the LH and POA groups differed significantly from controls. Again, no significant changes were seen at the two lower doses. At 60 minutes post injection, only the POA group was still consuming significantly less fluid. Again, the 50 μg dose was solely effective. Animals with LV cannulae never showed significant decreases in drinking at any dose used, nor did we observe any latency to first drink in any group.

These results implicate a central enlorphinergic mechanism regulating or otherwise influencing fluid intake behavior. Further, these sites are not necessarily within readily diffusable distance from the ventricles. It is clear that additional CNS sites be examined to further delineate the possible circuitry mediating this behavior. Supported by NIDA grant DA02234 to EAS.

164.12 GENETIC INFLUENCES ON INGESTIVE BEHAVIOR OF MICE WITH SEPTAL LESIONS. C. R. Goodlett,* P. J. Donovick, and R. G. Burright.* Department of Psychology, State University of New York at Binghamton, Binghamton, NY 13901.

Septal lesions in rats are reported to result in motivational changes, including increased intake of saccharin, and ad lib hyperdipsia with associated overdrinking following a hypovolemic challenge. However, the effectiveness of septal lesions in producing hyperdipsia in rats were unpredictable, and not explained on the basis of histological analysis. Changes in ingestive behavior have been less extensively studied in mice, though inbred strains are known to differ in their acceptance of saccharin (Fuller, 1974). Thus, we examined the effects of septal lesions in the genetically diverse Heterogeneous (HET) stock, as well as in the C57BL/6J, DBA/2J and AKR/J inbred strains, on feeding and drinking, and on the response to each of the following challenges: (1) saccharin acceptance; (2) cellular dehydration; (3) hypovolemia; and, (4) 24 hours of food deprivation.

Unlike rats, the groups of HET mice given septal lesions showed no evidence of ad lib hyperdipsia, not differing from surgical controls in their daily water intake. The lesioned mice also did not differ from controls in drinking elicited by saccharin, hypovolemia or cellular dehydration. However, during the 24 hour food deprivation, the septals drank significantly more water, and lost less bodyweight than did the controls. In further contrast to rats, HET mice with septal lesions increased their food consumption postoperatively to almost 20% more than control levels; this hyperphagia lasted the entire 50 day experimental period. Despite their increased food intake, the mice with septal lesions lost bodyweight after surgery, and maintained their bodyweight at about 90% of control levels. Only during the food deprivation, when septals drank more than controls, did the two groups have equal mean bodyweights.

Preliminary results from the inbred strains indicate that C57s drink more saccharin than DBAs and AKRs, as previously found. Septal lesions result in a large elevations of saccharin intake only in the DBAs, less elevations in the C57s, and no change in the AKRs. The hyperphagia and weight loss seen in the HETs was also found in all three inbred strains. These results emphasize the importance of genotype in the effects of limbic damage.

164.13 LONG TERM CHANGES IN NEURAL LOBES OF RATS WITH LESIONS OF THE PERIVENTRICULAR TISSUE SURROUNDING THE PREOPTIC RECESS (AV3V). J. R. Carithers and A. K. Johnson. Dept. of Veterinary Anatomy, Iowa State Univ., Ames, IA 50011 and Dept. of Psychology, Univ. of Iowa, Iowa City, IA 52242.

Rats with lesions in the AV3V region undergo acute adipsia and do not develop an appropriate antidiuretic response to the dehydration which results from their failure to drink. We have observed fine structural evidence that unreleased neurosecretory material is accumulated in neural lobes of lesioned rats during this acute phase. If the rats are maintained experimentally through this period, they resume drinking and become capable of excreting a concentrated urine in response to water deprivation. However, their drinking responses to treatment with angiotensin II or hypertonic saline are chronically impaired, and they exhibit chronic hypernatremia and hyperosmolality. Therefore, we have examined the effects of five days of water deprivation on the fine structure of neural lobes of rats which have received AV3V lesions or sham operations five weeks earlier.

In rats with sham lesions, five days of water deprivation led to depletion of neurosecretory granules and increased amounts of fine tubular smooth endoplasmic reticulum in axon terminals. In neural lobes of lesioned rats, there were more axons which contained accumulations of dense bodies, lamellated bodies and images suggestive of crinophagy and reorganization than were present in controls. Lesioned rats also had many axons which were filled with closely packed masses of neurosecretory granules, and these axons were usually ensheathed by attenuated processes of pituicytes. Such closely packed masses of neurosecretory granules were rare or absent in controls. After five days of water deprivation, changes which were qualitatively similar to those of control rats, but which involved fewer axons, were observed in neural lobes of lesioned animals. Axons which were packed with granules or appeared to be undergoing reorganizational changes were very noticeable after the reduction in neurosecretory material in water-deprived rats, but the number of such axons did not appear to have changed.

It appears that five weeks after AV3V lesions there are still axons which do not release their contents in response to appropriate stimuli for hormone release.

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164.14 DRINKING DEFICITS IN RATS AFTER LESIONS OF THE AV3V AREA. T. W. Gardiner* and E. M. Stricker (SPON: A. R. Caggiula). Dept. of Psych., Univ. of Pittsburgh, Pittsburgh, PA 15260.

In confirmation of previous observations (Buggy and Johnson, Am. J. Physiol., 233:R44, 1977), we find that ablation of the anteroventral wall of the third cerebral ventricle (AV3V region) abolishes the drinking response of rats to injection of hypertonic saline; nevertheless, most rats drink significant amounts within 10-12 hr after hypovolemia is produced by sc injection of polyethylene glycol (PEG) solution. However, further investigation of these phenomena indicates that these animals have not lost their capacity to respond to osmoregulatory stimuli, nor are their responses during hypovolemia unimpaired. In one series of experiments, caffeine (12.5 mg/kg, ip) injected together with 2 M NaCl (2 ml, sc) was found to restore drinking ($M \pm SD = 11.5 \pm 6.3$ ml/2 hr) in 8 of 10 lesioned animals that had failed to drink after injection of hypertonic saline alone. Caffeine alone was ineffective in eliciting drinking in either lesioned or control rats. Urine measurements indicated that the enhanced drinking was not secondary to an induced diuresis; in fact, drinking often preceded urine excretion by 30-60 min. In another series of experiments, 15 lesioned rats which had failed to drink following NaCl treatment were injected with 30% PEG (5 ml, sc). Four of these rats failed to drink during the subsequent 12 hrs. Among the 11 rats which did drink, 7 began drinking only after the light period ended (8 hr after the PEG treatment). Furthermore, this late, nocturnal drinking may not have been elicited by the hypovolemia, since most lesioned rats drank comparable amounts during a baseline period of food deprivation, when PEG was not given.

These findings parallel the results of recent investigations involving rats with lesions of the subfornical organ in which caffeine treatment was shown to restore drinking after PEG injection (Hosutt et al., J. Comp. Physiol. Psychol., 95:104, 1980). Collectively, they suggest that the drinking deficits that animals have after damage to these adjacent brain areas may not reflect disruptions specific to osmoregulatory or hypovolemic thirst but may instead be of a more general nature.

On the other hand, when 5 rats with AV3V lesions were tested for their feeding responses to 2-deoxyglucose (500 mg/kg, ip), food intake was found to be increased ($M \pm SD = 4.1 \pm 2.0$ g above baseline, 5 hr test) to an extent comparable to that observed in control animals. This preliminary finding suggests that the deficits of lesioned animals may be limited to drinking behaviors rather than including motivated ingestive behaviors in general.

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164.15 EFFECTS OF HEPATIC PORTAL INFUSIONS ON HYPOTHALAMIC NEURONAL ACTIVITY. W.-H. Tsai, F. C. Barone and M. J. Wayner. Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210.

Male rats were anesthesized with urethane and prepared for hypothalamic neuronal recording and peripheral vascular infusions. The mesenteric vein beside the descending colon was cannulated so that infusions only altered the portal circulation. The femoral vein was also cannulated so that infusions could enter the general circulation. Different solutions that were infused by both routes included: water; 0.15 M NaCl; 2.5 M NaCl; 1.7 M NaCl; 0.8 M NaCl; 5.0 M glucose and 5.0 M sucrose. Single neurons located in the lateral preoptic area (LPA) and lateral hypothalamus (LH) were studied. Neural activity was monitored continuously and the effects of 0.1 ml vascular infusions delivered at 0.04 ml/sec were determined. Responses were categorized according to changes in neuronal activity associated with the infusion of solutions via the two venous routes. Neurons that exhibited significant changes in discharge frequency when NaCl but not sucrose or glucose was infused were considered to be receiving inputs from sodium receptors. If NaCl effects were observed only via the mesenteric route, the cells were considered to be receiving inputs from hepatic sodium receptors. The majority of neurons that were effected by venous infusions were of this type. In some cases, NaCl concentration-response relationships for neurons could be established during mesenteric infusions. Other types of effects were observed and included neural responses associated with osmolality and pressure changes. In some cases, these effects were observed only via the mesenteric route, and cells were considered to be receiving inputs from hepatic osmo- and possibly baroreceptors. Very few neurons that were affected by venous infusions were of this type. Spinal cord and vagus nerve transections were made in order to determine the afferent pathways involved in the observed effects. In other experiments, animals were prepared for venous infusions and simultaneous recordings of electrocardiographic (EKG) and cortical electroencephalographic (EEG) activity and blood pressure were made. NaCl infusions by either mesenteric or femoral routes resulted in a short lasting desynchronization of EEG and a much longer lasting increase in blood pressure. These results indicate that synaptic input originating from sodium receptors associated with the hepatic circulation does modulate the activity of hypothalamic neurons and that these receptors probably participate in the regulation of drinking behavior by this mechanism.

164.16 **AMPHIBIAN FEEDING RESPONSES AS RELATED TO TEMPORAL AND REGIONAL CHANGES IN AMINO ACID PATTERNS OF THE CENTRAL NERVOUS SYSTEM.** **Claude F. Baxter, Jim W. Dole*, Ken H. Tachiki*, Betty B. Rose* and Roger A. Baldwin*. Neurochemistry Labs, VA Medical Center, Sepulveda, CA 91343, Dept. of Psychiatry, UCLA Sch. of Med. 90024 and Dept. of Biol., Calif. State Univ. at Northridge, CA 91330.**

Prey-seeking behavior and cerebral amino acid levels are altered during adaptation of the toad Bufo boreas to an altered osmotic environment. This amphibian seeks prey using both visual and olfactory cues. Changes in amino acid patterns and glucose in the olfactory bulbs, optic lobes, cerebral hemispheres and blood of toads in a hyperosmotic saline environment (HOE) have been studied and correlated with changes in feeding behavior.

Laboratory-adapted toads, feeding actively on mealworms, were used. They were exposed to HOE (410 ± 20 mOs) for from 2 to 28 days. Blood plasma osmolality equilibrated with environmental osmolality within hours after exposure to HOE. The qualitative changes in levels of most amino acids were similar for the three brain areas tested, but the % changes differed substantially for different brain areas. The time of peak levels for non-essential brain amino acids ranged from 4 to 8 days of HOE.

When toads in HOE, feeding on mealworms (F) were compared with toads that did not feed (NF), significant biochemical differences between F and NF were observed. The initial elevation of blood glucose, in response to HOE, was more rapid in toads that became NF. Some putative neurotransmitter amino acids were significantly higher in specific brain areas of NF as compared to the same brain areas in F, irrespective of time in HOE: i.e., glutamate in the cerebral hemisphere ($p < .025$), glycine in optic lobe ($p < .025$) and aspartate and glutamate in olfactory bulb ($p < .025$ and $p < .005$, respectively). Whereas most non-essential amino acids in brain tissues were higher in NF than in F, the reverse was true for many essential amino acids.

Amino acid levels in some areas of brain appeared to be correlated with aspects of feeding behavior. Strike accuracy (the number of successful strikes at prey, expressed as a % of total strikes) was correlated with critical levels of several amino acids in specific brain areas. For example, a 50% reduction of strike accuracy appeared correlated with phenylalanine increases of approximately 0.01 µmol/g in olfactory bulb, optic lobe and cerebral hemisphere. This represents only 6.3, 8.5 and 15% of the peak increases of this amino acid observed in brains of toads kept for 6 days in HOE. These results suggest that small regional changes in neurotransmitters or their precursors may be linked to changes in feeding behavior. (Supported by the Medical Research Service of the Veterans Administration.)

164.17 DISTURBANCE IN WATER REGULATION IN PSYCHIATRIC PATIENTS.
W.B. Lawson, C.N. Karson, J.E. Kleinman, K.F. Berman, L.B. Bigelow and R.J. Wyatt* (SPON: C. Duncan-Johnson). Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032 and National Institute of Health, Building 31, Room 4C-35, Bethesda, Maryland 20205.

Disturbances in water regulation have long been noted in psychiatric patients. Such a disturbance is manifested by polydipsia, and secondary peripheral consequences including polyuria, hypertension, and hyponatremia which may lead to seizures and death. In order to assess the prevalence of this disorder, urine output was measured in psychiatric inpatients admitted to our research ward over the past three years. Urine volume was found to be a reliable and quantifiable measure of water intake in these patients.

Results: Twenty-four hour urine volumes were measured in 58 chronic psychiatric male and female patients, 51 of whom were diagnosed as schizophrenic by RDC and DSM III. These patients, when they were at least six weeks medication free (MF), excreted significantly more urine than 29 normal controls (mean = 2345.48 ml, SE = 371.86 vs. mean = 1303.14 ml, SE = 116.85; Analysis of variance: F = 5.51, p .006, post hoc testing by LSD = p .04). This was also found to be the case when they were on neuroleptic medication (MP), usually haloperidol .4 mg/kg, at least six weeks (mean = 2935.87 ml, SE = 380.99, LSD = p .002). Twenty patients who participated in both the MF and the MP treatments excreted significantly more while on medication (mean = 2742.35 ml, SE = 551.88 vs. mean = 3720.60 ml, SE = 690.26, t = 2.12, p .04).

Conclusion: Disturbance in water regulation in psychiatric patients is more common then is generally thought, and may be exacerbated by antipsychotic medication. The possibility of a relationship between mental illness and the biochemistry of central thirst mechanisms will be discussed. For example dopaminergic and endogenous opiate systems have been implicated in the etiology of schizophrenia and the regulation of thirst.

165.1 EFFECTS OF PIMOZIDE ON SACCHARIN AND SUCROSE CONSUMPTION. D. Sandberg*, M. Vaillancourt*, R. Wise and J. Stewart (SPON: F. Ervin). Dept. of Psychology, Concordia Univ., Montreal, Canada H3G 1M8.

The role of dopamine in mediating the rewarding property of sweet solutions was evaluated by comparing the effects of pimozide, a dopamine receptor blocker, on the consumption of sodium saccharin and sucrose solutions.

In the first experiment, nondeprived male rats were given six, eight-hour test sessions, separated by at least two days, during which they were offered a choice between water and one of six sodium saccharin solutions (.009%, .028%, .083%, .25%, .75%, and 2.5%; w/v). The order of presentation of the saccharin solutions was random and the specific concentrations were chosen so as to yield a biphasic relationship between saccharin concentration and intake. Separate groups were injected intraperitoneally with either .5 or 1.0 mg/kg pimozide or the tartaric acid vehicle four hours prior to the test. Up to a point, intake of the saccharin solution increased with elevations in the solution concentration. Further increases in the solution concentration resulted in decreased intake. Although the mean values for saccharin intake of the two pimozide groups were consistently below those of the control group, group differences did not achieve statistical significance.

In the second experiment, the effects of pimozide on consumption of sucrose solutions were assessed. The procedure was identical to that used in Experiment 1 with the exception that a series of six sucrose solutions (.68%, 1.71%, 3.42%, 6.81%, 17.1%, 34.2%; w/v) replaced the six saccharin solutions. Concentrations of the solutions were chosen to produce an inverted-U function similar to that observed when saccharin was used as the test solution in Experiment 1. Control group animals consumed 2.5 times more of the optimal sucrose solution than had previously been observed with the most preferred concentration of saccharin. Pimozide suppressed intake of sucrose in a dose-dependent fashion but only at those concentrations that comprised the ascending limb of the inverted-U function. These findings are discussed in relation to both reward- and performance-deficit hypotheses of pimozide action. Some similarities between the effects produced by pimozide and cholecystokinin on consummatory behavior are also discussed.

165.2 ESTRUS AND DIESTRUS HORMONAL CONDITIONS INFLUENCE THE FEEDING RESPONSE TO 2-DEOXY-D-GLUCOSE. Lois J. McDermott and Denise E. Jorgensen.* Biopsychology Lab. University of Colorado, Colorado Springs, CO 80933.

The food intake of female albino rats was measured 4 hours following injections of 2-deoxy-d-glucose (2DG) (250 or 450 mg/kg, i.p.) or isotonic saline (which constituted a baseline for comparison). Females with cycling ovaries (NORM, n=18) were tested with 2DG the mornings of estrus (EST) and diestrus (DIEST). Ovariectomized (OVX, n=18) females were tested the day following injections of estradiol benzoate (20.0 µg/rat, s.c.) and progesterone (2.5 mg/rat, s.c.) (EST-H) or sesame oil (DIEST-O). These treatments were employed as models of the hormonal conditions of EST and DIEST, respectively. Prior to injections of 2DG or saline vaginal smears were examined to confirm the hormonal condition of the NORM and OVX groups. Daily food intake of half of the OVX group (OVX-R, n=9) was restricted to keep the body weight of these animals similar to the NORM group the remaining subjects of the OVX group (OVX-AL, n=9) were allowed ad libitum access to food and exhibited significantly elevated body weights relative to the NORM and OVX-R groups. Subjects were tested 3 times with each dose of 2DG or saline under both hormonal conditions with at least 4 days between tests.

Injections of 2DG produced a significant increase in food intake relative to baseline in the NORM and OVX groups during both the EST/EST-H and DIEST/DIEST-O conditions (NORM/OVX, respectively). The response to 2DG of OVX groups during DIEST-O was significantly larger than that obtained during EST-H. (A difference in response to 2DG during EST and DIEST conditions in the NORM group was observed but did not achieve customary levels of significance.) Although baseline intake for both NORM and OVX groups was suppressed during EST/EST-H relative to DIEST/DIEST-O, this difference was not sufficient to account for the magnitude of the difference that was observed in the response to 2DG during the different hormonal conditions.

The magnitude of the feeding response to 2DG relative to baseline was not only a function of the hormonal conditions during testing (EST-H vs. DIEST-O) but was also a function of some concomitant of the body weight of the animal. The OVX-AL group exhibited both a larger response to 2DG relative to baseline as well as greater body weight than the NORM group. Further the difference between the response to 2DG under the EST/EST-H vs. DIEST/DIEST-O conditions was larger for OVX females than NORM females as was the body weight of the OVX group.

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165.3 DISSOCIATION OF FOOD INTAKE AND PLASMA BETA-ENDORPHIN IN TUMOR-BEARING RATS AND IN DEXAMETHASONE-TREATED RATS FOLLOWING 2-DEOXY-GLUCOSE. G. K. W. Yim, M. T. Lowy*, J. M. Davis*, D. R. Lamb* and P. V. Malven. Dept. Pharmacol./Toxicol., Physical Educ., Animal Sci., Purdue Univ., W. Laf., IN 47907.

Food intake (FI) following administration of 2-deoxyglucose (2-DG) is known to be decreased in normal rats injected with dexamethasone (DEX) and in Sprague-Dawley rats bearing hindlimb implants of the Walker-256 carcinosarcoma (tumor-bearing rats; TBR). Since these TBR are known to have enlarged adrenal glands and elevated glucocorticoid levels, they may be comparable to DEX-treated normal rats in that both may experience glucocorticoid-induced inhibition of pituitary beta-endorphin (β-EP) release. Our previous work showed that plasma β-EP was increased during various hyperphagic states (i.e. nocturnal hyperphagia and daytime hyperphagia induced by food deprivation, acute exercise or 2-DG treatment). The present study examined the relationship between basal and 2-DG induced levels of plasma β-EP and FI in normal DEX-treated rats and in TBR. β-EP was quantified in silicic acid extracts of rat plasma using radioimmunoassay procedures described previously. Elevated concentrations of plasma β-EP were observed following 2-DG in agreement with earlier results, and DEX pretreatment (400 µg/kg and 200 µg/kg at 24 and 2 hr prior to sampling) blocked the 2-DG induced elevation (509 ± 16 vs 176 ± 14 pg/ml). However, DEX did not reduce FI during the 1 hr following 2-DG (3.0 ± .3 vs 3.2 ± .3 gm). In TBR, plasma β-EP did not fluctuate between day and night as in controls. Daytime injection of 2-DG raised plasma β-EP in TBR (555 ± 59 pg/ml) to the same concentration as in non-tumor controls (509 ± 16 pg/ml). However, FI following 2-DG was reduced by 35% in TBR as compared to non-tumor controls.

In summary, DEX decreased plasma β-EP in non-tumor rats without affecting 2-DG induced eating during the first hour. Untreated and 2-DG treated TBR were anorexic but their plasma concentrations of β-EP were not deficient. These results illustrate two situations where plasma β-EP and FI could be experimentally dissociated. (Supported in part by ACS grant CH 194, NIH grant TG GM07095, PHS grant DA-2661, and a Purdue Research Foundation fellowship.)

165.4 OPPOSITE EFFECTS OF YOHIMBINE AND CLONIDINE ON THE FOOD INTAKE OF GENETICALLY OBESE (obob) MICE. M. Beales*, M.F. Callahan, and G. A. Oltmans. Dept. of Pharmacology, Chicago Med. School, N. Chicago, IL 60064.

The obesity syndrome of the genetically obese mouse, obob, is characterized by behavioral, physiological, and hormonal abnormalities. Several CNS abnormalities have also been identified, including increased hypothalamic (HT) norepinephrine (NE) levels, increased number of HT alpha-adrenergic receptors, and decreased HT NE release. Since application of NE agents to the HT can modify feeding, it is possible that defects in endogenous NE systems could result in hyperphagia. Previous work has indicated that amphetamine, which activates central catecholamine systems, reduces food intake in obob mice. In the current study we examined the effects of the alpha-2 receptor agonist clonidine and the alpha-2 receptor blocker yohimbine on food intake in obob and lean mice.

All animals were adapted to a 6-hour feeding schedule until a stable baseline food intake was established (about 3 weeks). Thirty minutes before food was presented animals were injected with yohimbine, clonidine, or vehicle and placed in individual cages. Food intake was measured after 1, 3, and 6 hours of food access. Relatively low doses of both drugs were used in an attempt to affect primarily alpha-2 receptors. Both drugs had their greatest effects on food intake during the first hour of food access. Yohimbine (1 to 3 mg/kg) produced a dose-dependent decrease in the food intake of obob mice (see table). Higher doses (4 to 5 mg/kg) were required to decrease the food intake of lean mice. A low dose of clonidine (100 µg/kg) increased the food intake of obob mice (140% of control). Higher doses of clonidine (200 to 500 µg/kg) produced a dose dependent decrease in the 3 hour food intake of both lean and obob mice.

Effect of Yohimbine on 1 Hr. Food Intake in obob and Lean Mice

Food Intake[a]	Yohimbine Dose (mg/kg)				
	1	2	3	4.6	5.0
lean	90	79	80	72*	59*
obob	92	75*	59*	--	--

[a]Food intake is expressed as a % of the vehicle treated lean or obob control.

*Differs significantly from vehicle treated controls, $p < .05$.

These results suggest that obob mice may be more sensitive than their lean littermates to the effects of alpha-2 adrenergic drugs on food intake (eg., anorexia or hyperphagia). This may reflect an abnormality in the presynaptic alpha-receptors of the obob mutant, and such an abnormality might result in decreased NE release. The failure to release adequate amounts of NE may result in failure to limit food intake to caloric requirements. (Supported by NS 15600).

165.5 CHOLECYSTOKININ:EFFECTS OF DURATION AND DOSE ON FEEDING BEHAVIOR, INSULIN AND GLUCOSE IN RHESUS MONKEYS. Bonnie L. Metzger* and Barbara C. Hansen*. (SPON:P.Coyle). Dept. of Physiology, University of Michigan, Ann Arbor, MI 48109.

Exogenous cholecystokinin (CCK-8) produces feeding cessation in human and non-human animals. In these studies, we examined the dose response and time course of intravenous (IV) synthetic CCK-8 on eating behavior and associated post-absorptive insulin and glucose responses to a mixed meal in male monkeys following an overnight fast. Four monkeys (Macaca mulatta) weighing 6-9 kg were fitted with IV cannulae. Saline controls were used and doses randomized. A minimum of 16 hours separated experiments. Feeding behavior was measured by the size and length of each meal, the length of subsequent inter-meal intervals (IMI), number of meals and total daily kcal intake. Plasma insulin (IRI) and glucose were monitored for 120 min following feeding start. In experiment 1, CCK-8 IV infusions were initiated at the start of voluntary feeding and continued for 6 min at a rate of 15, 30, 60 or 120ng/kg/min. Rapid suppression of feeding occurred within 2.5 min,however, the effective dose varied across monkeys ranging from 30 to 120ng/kg/min. Each CCK-8 enhanced feeding interruption lasted <20 min. Because the effects of the 6 min infusion were of short duration, experiment 2 was designed to test the effect of continuous infusions. Using doses approximating anticipated physiological levels (2,4 or 8ng/kg/min), infusions were started 30 min prior to and continued through the animal's usual 8 hour feeding period. Only the 8ng/kg/min dose consistently reduced total daily intake ($p<.0001$). The decrease in intake was the function of diminished kcal consumption during the 1st ($p<.001$) and 2nd ($p<.05$) daily meal. There was no CCK-8 enhanced delay in the onset of subsequent meals. The minimal effective dose ranged from 4ng/kg/min (n=2) to no response during 8ng/kg/min in 1 animal. Across all experiments only CCK-8 doses >15ng/kg/min delayed both IRI and glucose rises following the meal relative to saline controls ($p<.05$). We conclude that IV exogenous CCK-8 reduces usual meal size and length with maximal effects in the early part of the feeding period. The large dose (>30ng/kg/min) CCK-8 satiety effect is extremely rapid and brief suggesting that this effect is not dependent upon associated stomach distention or the passing of nutrients into the duodenum. Further, the response delays of IRI and glucose induced by CCK-8 do not correlate with feeding interruptions and imply that neither IRI nor glucose alterations mediate CCK-8 enhanced satiety.

165.6 THE EFFECT OF CHRONIC MORPHINE ADMINISTRATION ON MACRONUTRIENT SELECTION IN THE RAT. R. Ottaviani* and A. Riley*. (SPON: C. F. Tyner). Psychopharmacology Laboratory, The American University, Washington, D.C. 20016.

Recently, Marks-Kaufman and Kanarek (Pharmac. Biochem. Behav. 12:427-430, 1980), reported that the acute administration of morphine sulfate selectively affected the rat's intake of a range of macronutrients. For example, morphine suppressed the consumption of carbohydrates and proteins throughout a six hour test. While fat consumption was also initially suppressed by morphine, by the end of the six hour sampling period, fat intake was elevated above control values.

Because morphine also produces marked catatonia at high doses (Riley, Ortuno, Hoffman, Siemon, & Heft, Neurosci. Abst. 6:783, 1980), it is quite possible that the initial suppression of feeding of all macronutrients reflects this interfering behavioral suppression. The subsequent inverse in fat intake, in turn, may reflect a simple compensation for lost calories and not a direct stimulating effect of fat intake by morphine. If the delayed fat intake is compensatory, as tolerance develops to the morphine-induced catatonia, there should be no initial suppression of fat intake and similarly no compensatory increase. The following experiment examined the effects of chronic morphine exposure on macronutrient selection in the rat.

Following a distilled water injection baseline period of seven days, during which time the macronutrient intake of fats, carbohydrates and proteins was assessed for six hours each day, rats were injected with morphine sulfate or distilled water for 21 consecutive days and monitored daily for macronutrient selection. While both groups consumed approximately 5.5 g of food during baseline, morphine significantly suppressed overall food consumption over the 21 days of injections. In addition to affecting total intake differentially, morphine selectively affected macronutrient selection upon acute and chronic administration. Throughout injections, the percentage of fat intake for morphine-injected rats (75%) was significantly higher than that for control subjects (41%), i.e., morphine-injected rats substantially preferred fats to carbohydrates (or proteins). This difference was evident even on Day 21 when catatonia had functionally dissipated and when controls and drugged animals showed no initial differential suppression of fat intake following injections.

These data suggest that morphine selectively alters macronutrient selection in the rat with this effect due to a direct action of morphine on feeding not to a compensatory intake.

165.7 EFFECTS OF CENTRAL INJECTIONS OF NEUROTRANSMITTERS AND DRUGS ON FREELY-FEEDING RATS, J. Grinker, C. Marinescu* and S.F. Leibowitz. Rockefeller University, New York, New York 10021.

Meal pattern analysis allows the evaluation of neurotransmitter and drug effects on spontaneously feeding animals. The feeding changes induced by central injections of norepinephrine (NE), amphetamine (Amph) - a releaser of NE and dopamine, and chlorpromazine (CPZ) - a dopamine receptor antagonist, were compared.

Rats were chronically implanted with 23 gauge cannulae aimed at the perifornical hypothalamus or the paraventricular nucleus. Adult male Sprague Dawley rats (n=6) received paraventricular nucleus injections of NE (20nmoles) or vehicle (normal saline-NS). Injections of both NE or NS were administered for five days at 10 A.M. in a counterbalanced design. Additional groups received perifornical hypothalamic injections of Amph (150nmoles) or vehicle in the A.M. or CPZ (100nmoles) or vehicle in the P.M. Injections were administered for each substance and for vehicle control for at least five days. Rats were maintained on a diet of 80% lab chow and 20% dextrose and a 12/12 light-dark cycle. Meals were defined as minimum feeding bout of 10 licks with an intermeal interval of 15 minutes. Meal pattern data were collected on a PDP 8 computer connected to solid food lickometers (Strohmayer and Grinker, Physiol. Behav. 24:789, 1980).

The two drugs produced different behavioral profiles. Amph decreased the duration, size and latency of the first meal and increased the rate of a subsequent meal. CPZ increased the duration, rate, and size of the first meal and also lengthened the intermeal intervals. In contrast, NE increased the size and duration of the first meal as well as the rate of eating but failed to have sustained effects on meal frequency.

Ritter and Epstein (Proc. Nat. Acad. Sci., 72:(9) 3740, 1975) have also reported that hypothalamic injections of NE in the freely-feeding rat increased meal size but did not change meal frequency. We now report that Amph and CPZ have opposite effects on the microstructure of feeding. These data suggest that meal analysis can provide important information about drug and metabolic interactions in the control of normal feeding. (Supported in part by NIH 22879 and AM 27980).

165.8 BRAIN SEROTONIN AND TRYPTOPHAN IN GENETICALLY OBESE AND DIABETIC MICE. Joan F. Lorden and Joanne P. Steves.* Department of Psychology, University of Alabama in Birmingham, Birmingham, AL 35294.

Obese (ob/ob) and diabetes (db/db) mice display syndromes characterized by obesity, hyperglycemia and hyperinsulinemia. The primary cause of the syndrome has not been identified for either mutant; however, assays of central monoamines have revealed elevated levels of hypothalamic norepinephrine in both cases (Lorden et al., Brain Res., 1975, 96, 390-4) suggesting the possibility of a central nervous system defect. Recent studies (Garthwaite et al., Endo., 1979, 105, 1178-82) have also indicated that 4-5 mo old ob/ob mice have elevated levels of central serotonin (5HT), a proposed mediator of satiety. Since the elevated insulin levels of the db/db mouse might be expected to have a similar effect on 5HT in that mutant, we assayed the brains of 7 wk old, female, ob/ob (C57BL/6J-ob) and db/db (C57BL/KsJ-db) mice and lean controls for 5HT and tryptophan (TRP) using a fluorometric technique. The mice were fed ad lib and food was removed two hours before sacrifice. Both the ob/ob and db/db mice were significantly obese and hyperglycemic at sacrifice. In the ob/ob mice, TRP and 5HT levels were elevated in the brainstem and telencephalon. No significant differences in 5HT levels could be found in the db/db mice, although TRP was greatly reduced. This may indicate a decreased rate of 5HT turnover in diabetes mice.

In a second experiment ob/ob, db/db and lean control mice from each background strain were fasted overnight. Ninety to 120 min prior to sacrifice, the mice were given oral infusions (.3cc) of either water or 2M glucose. Fasted ob/ob mice were hyperglycemic and hyperinsulinemic in comparison with lean controls and the glucose meal increased blood glucose and plasma insulin in both groups. However, increases in 5HT and TRP levels were seen only in the ob/ob mice. In the fasted db/db mice, TRP levels were lower than those of lean (db/m) controls; however, 5HT levels did not differ. As in the ob/ob mice, a glucose meal had a greater effect on TRP and 5HT levels in db/db mice than in lean controls. The differential effect of glucose in mutant and normal mice is most likely due to increased insulin release by the mutants. The results suggest some basic differences in the neurochemistry of these mutants, despite similarities in the syndromes. The significance of these differences for the obese and diabetes syndromes remains to be assessed; however, widespread disturbances in central monoaminergic systems may be important factors in the endocrinological disorders seen in these mice. (Supported by NINCDS grant NS 14755).

165.9 FOOD DEPRIVATION, NALOXONE AND PLAY. S. Siviy*, G. Davies* N. Najam* J. Rossi III* and J. Panksepp (SPON: Z.M. Nagy). Dept. of Psych., Bowling Green State Univ., Bowling Green, OH 43403

It is well established that naloxone reduces both feeding and social play in rats. In analyzing the interactions between feeding and play in young Long-Evans rats, we observed that 24 hrs of food deprivation reduces social play by 57%. The ability of hunger to reduce play is promptly reversed by a single meal. Accordingly, play may be used as a sensitive behavioral indicator of normal satiety processes. For instance, if a pharmacological substance reduces food intake by inducing normal satiety, it should also cause hungry animals to play more. The ability of satiety to invigorate social play could serve as a highly stringent criterion for specification of normal satiety factors in the body. Since the reduced feeding following naloxone has been suggested to reflect normal satiety, we evaluated the ability of the drug to reverse deprivation-induced reduction in play. Rather than restoring play, naloxone (1 mg/kg) further reduced it. To further clarify how naloxone modulates food and social motivation in a single situation, young rats (14-16 days old) were allowed to choose between dirty home bedding on one side of a test chamber and their anesthetized mother upon clean bedding at the other end, latency to begin suckling being measured. Rather than slowing suckling latency, as would be predicted by a satiety hypothesis, naloxone-treated animals latched onto a nipple in less than half the time of control animals (44 vs 136 sec for naloxone and saline pups, respectively), suggesting naloxone had amplified an internal state of social need. Also, in preweanling rats, naloxone did not reduce food intake (as measured by weight gain following one hour of suckling) Indeed, young rats treated chronically with naloxone (3-26 days of age, 5 mg/kg, three times a day) grew faster than controls.

These data suggest that appetite reduction evoked by opiate receptor blockade may be similar to anorexia induced by social separation. Furthermore, the ability of food deprivation to reduce play, provides a compelling behavioral end-point for evaluating the normality of food intake reductions induced by drugs and hormones.

165.10 NALMETRENE DECREASES FOOD AND WATER INTAKE AND BODY WEIGHT GAIN IN ZUCKER OBESE AND LEAN RATS. C.L. McLaughlin, C.A. Baile and R.R. Tuttle*. Sch. Vet. Med., Univ. of Penn., Kennett Sq., PA 19348 and Key Pharmaceuticals, Inc., Miami, FL 33169.

Opiates may play a role in the control of food intake since administration of opiates increase, while administration of opiate antagonists decrease food intake. There is evidence that obese rats and mice have increased concentrations of opiates and are more sensitive than normal-weight rats and mice to the effects of opiate antagonists on food intake. In these experiments food and water intake, body weight gain and feeding behavior responses to a new opiate antagonist, nalmetrene (NM) were measured in Zucker obese and lean rats. In experiment 1, male Zucker obese (548±6 g) and lean (415±10 g) rats were administered 0, .03, .06 or .125 mg/kg NM twice daily for 1 wk (n=5 obese and 5 lean per treatment). Average daily ad libitum food intakes were decreased relative to pretreatment intakes by all treatments in lean (Δ=.7±.5, .3±.6 and -.1±.4 vs 3.6±1.1 g, $p<.01$, ANOVA) but not obese rats. Weight gains were less in treated than control lean rats (0±.2 vs .9±.2 g/day, non-paired-t=2.59, $p<.01$), but water intakes were not affected in obese or lean rats. In experiment 2, Zucker obese (477±6 g) and lean (387±9 g) rats were administered 0, .125 or .25 mg/kg NM twice daily for 21 days (n=3 males and 3 females per phenotype per treatment). Only in obese rats was food intake decreased by .125 mg/kg NM the first 2 wks (Δ=-3.0±1.1 vs .5±1.0 g, ANOVA, $p<.05$) and rate of weight gain was decreased by .125 and .25 mg/kg NM the first wk (1.2±.7 and 1.5±.3 vs 3.3±.7 g/day, $p<.05$, ANOVA). Daily food and water intakes and rate of body weight gain were not different from control after 3 wks of NM treatment, indicating development of tolerance. In experiment 3, feeding behavior response to 7-day administration of 0 or 1 mg/kg NM was measured. Ten male Zucker obese rats (326±14 g) were treated at the onset of the dark portion of the 12-hr light-dark cycle and allowed to bar press for food for 14 hrs. Compared with the pretreatment wk NM decreased 14-hr food intakes (28.0± 1.2 vs 31.3±1.6 g, t=3.27, $p<.01$) by decreasing average meal size (3.2±.3 vs 3.7±.3 g, t=3.64, $p<.003$); however, the effects occurred only in the first 3.5 hrs since feeding behavior 3.5-14 hrs was not affected. The decrease in food intake 3.5 hrs after treatment was similar on the first and last days (9.6±.9 vs 7.8±1.0 g) indicating lack of development of tolerance after 7 days. Average daily weight gains and water intakes were also decreased (3.2±.3 vs 4.5±.4 g, t=3.13, $p<.006$ and 37±2 vs 47±3 ml, t=3.59, $p<.01$), although the decrease in water intake was proportional to the decrease in food intake. Thus, chronic administration of the new opiate antagonist NM can decrease food intake and body weight gain in Zucker obese and lean rats and the effect is primarily on average meal size.

165.11 ABDOMINAL VAGOTOMY REDUCES THE DIPSOGENIC BUT NOT THE ANOREXIC ACTION OF SYSTEMIC SEROTONIN IN RATS. K. J. Simansky, K. A. Bourbonais*, and G. P. Smith. Dept. Psychiatry, Cornell Univ. Medical College, White Plains, NY 10605

Systemic administration of serotonin inhibits food intake (Pollock & Rowland, Pharmacol.Biochem.Beh., 1981, 15, 179) and produces drinking (Kikta et al, Pharmacol.Biochem.Beh., 1981, 14, 889) in rats. We determined the effects of abdominal vagotomy on the anorexic and dipsogenic actions of six doses of serotonin (.25-5.6 mg/kg sc) injected 12 min before a liquid milk diet was provided to male rats deprived for 17h of milk but not water. Serotonin reduced 30 min food intake in the controls (n = 6) in a dose-dependent manner. Vagotomy (n = 5) did not significantly alter the inhibition of food intake by these doses of serotonin. For example, the apparent threshold dose of 0.5 mg per kg reduced food intake by 30 ± 8% in controls and 46 ± 13% in vagotomized rats. In comparison, cholecystokinin octapeptide (4 mcg/kg ip) failed to inhibit feeding in these vagotomized rats (-14 ± 14%) while decreasing food intake by 56 ± 9% in the laparotomized controls. This effect of vagotomy on cholecystokinin-induced satiety replicates our earlier work (Science, 1981, 213, 1036) and, furthermore, suggests that the normal anorexic effect of serotonin after vagotomy was not an artifact of the different baselines for 30 min food intake (14.4 ± 1.5 ml, controls vs. 6.2 ± .4 ml, vagotomy; $p < .01$).

In contrast to the above, vagotomized rats drank less than controls ($p < .05$) during the 60 min after presentation of food. For example, controls drank 6.8 ± .9 ml after 2.0 mg/kg serotonin while vagotomized rats drank 2.6 ± .9 ml ($p < .05$). The baselines did not differ (3.1 ± .3, controls; 2.8 ± .7, vagotomy). When food was not provided under these conditions, controls drank 8.3 ± 1.5 ml during the 132 min after injection and vagotomized rats drank 3.8 ± .6 ml ($p < .01$).

These results demonstrate that the dipsogenic, but not the anorexic, action of systemic serotonin is mediated, in part, by the abdominal vagus nerve in the rat.

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165.12 EFFECTS OF IMIDAZOLE-4-ACETIC ACID (IMA) AND CHLORDIAZEPOXIDE (CDP) ON DRINKING BEHAVIOR OF THIRSTY RATS. I. P. Day* AND B. BEER. Dept. CNS Research, Medical Research Division of Amer. Cyanamid, Lederle Laboratories, Pearl River, NY 10965

IMA, a metabolite of histamine, is a pharmacologically active agent. Its presence in the brain suggests a role in the functioning of the central nervous system. Based on opposite effects of IMA and CDP on conditioned emotional responses in rats, it was proposed that IMA has a causal relationship in the production of anxiety states (Clody, et al. 80th Annual Convention, APA, 825, 1972). CDP, as well as other benzodiazepines, disinhibits behavior suppressed by a variety of stimuli. Benzodiazepines increase both regular drinking and feeding behavior and drinking of unknown or adulterated solutions (Poschel, B., Psychopharmacologia 19:193, 1971).

We examined the interaction of IMA and CDP in drinking behavior in rats. Naive rats were deprived of water for 48 hours and placed in single cages the day before the experimental session. Drugs were administered intraperitoneally at a volume of 0.1 ml/100g. Graduated Richter tubes containing tap water or quinine solution were presented immediately after treatment or 30 minutes after treatment.

IMA decreased drinking of tap water in a dose-dependent manner. The lowest dose causing a significant effect was 30 mg/kg i.p. Maximum suppression of drinking was observed when the drinking tubes were presented immediately after the injection of IMA. CDP at 4, 8 and 12 mg/kg i.p., injected 30 minutes prior to IMA had either no effect or enhanced the IMA-induced suppression of drinking. The interaction of IMA and CDP was examined in an aversive paradigm where a quinine-adulterated solution was presented to water-deprived rats. Drinking behavior was decreased with increasing concentration of quinine. CDP increased both drinking of tap water and water adulterated with quinine. IMA treatment caused further suppression of drinking of quinine solution in a dose-dependent manner. CDP at 4 and 8 mg/kg i.p. administered 30 minutes prior to IMA had no effect on IMA-induced suppression of drinking behavior.

The mechanism of IMA-induced suppression of drinking behavior in rats is not clear. This effect is not antagonized by CDP. Both compounds, IMA and CDP, decrease motor activity and cause ataxia. Ataxia by itself does not decrease drinking behavior, as seen with CDP. The ED_{50} for ataxia, measured in a rod-walking test in rats, was 7 mg/kg p.o., which is about the same as the dose significantly increasing drinking. The ataxic ED_{50} of IMA was 97 mg/kg i.p. and the doses suppressing drinking behavior used in this experiment were 30 and 60 mg/kg i.p. IMA and CDP at the doses used in this study both decreased motor activity in rats by about 30% in comparison with controls.

165.13 BRAIN MONOAMINE CHANGES ASSOCIATED WITH BURN-INDUCED ANOREXIA. Y. Berlatzky*, W. T. Chance, F. van Lammeren* and J. E. Fischer* (SPON: A. Mathieu). Department of Surgery, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.

Burn trauma induces a hypermetabolic state, the degree of which is directly related to the severity of the burn. Although this hypermetabolism has been related to increased catecholamine activity, no direct measurement of brain levels of norepinephrine (NE) or dopamine (DA) have been reported in a appropriate animal burn model. In preliminary studies we observed that a 30% surface burn in guinea pigs resulted in significant anorexia in addition to hypermetabolism as well as increased whole brain levels of DA and the serotonin (5-HT) metabolite, 5-hydroxyindoleacetic acid (5-HIAA). Therefore, we investigated monoamine changes in 10 brain areas of burned (B, n = 10), pair-fed (PF, n = 9) and freely-feeding (FF, n = 8) guinea pigs. Burn trauma was induced in anesthetized guinea pigs by a 30 sec open flame (kerosene-soaked gauze applied to the shaved back). Burned and FF animals were provided food (30% casein) and water ad lib. Each animal of the PF group was allowed water ad lib. and had access only to the amount of food consumed during the preceeding day by the B group. Although body weights decreased daily following the burn, food intake rebounded 3 days afterward and then significantly declined to less than 60% of control values. Burned and FF animals were sacrificed on day 6, with the PF animals being decapitated on day 7. Brains were rapidly dissected into 10 areas and frozen in liquid N_2. Amino acids were measured in plasma, while brain regions were homogenized in formic acid-acetone for quantification of monoamines by HPLC. Threonine, valine, cysteine, leucine, tyrosine (Tyr), phenylalanine, ornithine, lysine, arginine and 3-methylhistidine were elevated in B animals' plasma, while glutamate and glycine were decreased. Thus far, 4 of the 10 areas have been analyzed for monoamine changes. Tryptophan (Tryp), Tyr, and 5-HT were significantly increased in the hypothalamus of B and PF animals, while 5-HIAA was elevated only in the B group. In the septal area, Tryp, Tyr and DA were elevated in B and PF animals, while the DA metabolite, 3-methoxytyramine (3-MT) was increased in PF as compared to B animals. The amygdala exhibited increased Tryp and Tyr and decreased homovanillic acid (HVA) in B and PF animals, while 3-MT was decreased in B as compared to PF guinea pigs. Within the mesencephalon, Tryp was elevated in B and PF animals, 5-HIAA, dihydroxyphenylacetic acid and NE were increased in B animals, while HVA was decreased in PF guinea pigs. These data suggest that hypothalamic serotonergic systems and dopaminergic systems in the amygdala and septal areas may be important in the mediation of burn anorexia, since B and PF groups exhibited divergent responses in monoamine metabolites in these areas.

165.14 NORMALIZATION OF BRAIN INDOLEAMINES IN ANORECTIC TUMOR-BEARING RATS BY FORCED ENTERAL FEEDING. F. van Lammeren*, W. T. Chance, H. I. Karlberg* and J. E. Fischer* (SPON: M. von Meyenfeldt). Dept. Surgery, Univ. Cincinnati Med. Ctr., Cincinnati, OH 45267.

We have previously reported (Am. J. Surg. 143: 133, 1982) that rats bearing Walker (W) 256 carcinosarcomas exhibit increased brain levels of the serotonin (5-HT) precursor, tryptophan (Tryp), and metabolite, 5-hydroxyindoleacetic acid (5-HIAA) immediately prior to the onset of anorexia. In order to better investigate the relationship of nutrition to CNS monoamines in TB rats, the effects of supraoptimal enteral feeding of rats bearing W 256 tumors was studied. Tumors were induced in 13 immature, female, Sprague-Dawley rats by the im. injection (0.1 ml) of 5 X 10^4 W 256 cells. Similar rats were divided into pair-fed (PF, n = 8) and freely-feeding (FF, n = 8) control groups. Five days later, one group of TB (n = 6) rats was begun on enteral force feeding (40% solution of modified tube-feeding diet, ICN, Cleveland, OH) by gastric tube at 125% of the daily ad lib. (AL) intake of the FF group. Significant anorexia was observed in the AL-TB rats (n = 7) by day 6, with the rats being sacrificed on days 9 and 10 (PF). Brains were frozen in liquid N_2 prior to their homogenization in formic acid-acetone for quantification of monoamines by HPLC. Although plasma albumin levels were decreased in both groups of TB rats, the enteral feeding significantly restored concentrations towards normal. Plasma total Tryp was significantly decreased in both TB and PF groups, while free Tryp was significantly increased in both TB groups. Levels of brain Tryp were reduced in the enterally-fed TB rats as compared to all other groups. The AL-TB group exhibited significantly increased brain 5-HIAA, which was normalized by enteral feeding. Although norepinephrine was significantly decreased in AL-TB and PF groups, the enteral feeding also normalized this effect, suggesting a nutritional etiology. Levels of dopamine (DA) and dihydroxyphenylacetic acid were not changed across these groups. Other DA metabolites, however, were differentially affected. Thus, 3-methoxytyramine (3-MT), was significantly decreased in both TB groups and increased in PF rats, while concentrations of homovanillic acid (HVA) were significantly elevated in both TB groups and in the PF group. These results suggest that aberrations in DA metabolism may be more important in the mediation of cancer anorexia than the previously-reported indoleamine changes. Thus, 3-MT concentrations were differentially affected by the tumor and PF treatments. The absence of reversal of changes in both 3-MT and HVA by the enteral feeding schedule suggests that alterations in brain concentrations of these DA metabolites are not due to the effects of under nutrition.
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165.15 HYPERPHAGIA FOLLOWING INTRAHYPOTHALAMIC MICROINFUSIONS OF 5,7-DIHYDROXYTRYPTAMINE: EVIDENCE FOR A POTENTIAL SEROTONERGIC MEDIATION OF ESTROGENIC INFLUENCES UPON FEEDING. D. J. Steel*, R. J. Waldbillig* (SPON: Vernon J. Odom) Department of Psychology, University of Florida, Gainesville, Florida 32611

Recently this laboratory has presented evidence indicating that in female rats, anatomically restricted and neurochemically selective depletions of forebrain serotonin result in a high-fat diet-contingent hyperphagia and obesity (Waldbillig, Bartness and Stanley, JCPP 95: 391, 1981). Although this earlier study did not attempt to relate food intake to estrous cycle stage, an inspection of the raw data had suggested a failure of serotonin-depleted rats to show the reductions in food intake typically attributed to the estrous stage of the cycle. The purpose of the present study was to directly examine the effects of forebrain serotonin depletions on estrous cycle related variations in food intake.

Two groups of 10 Long-Evans female rats, matched with regard to body weight, received intraperitoneal injections of desmethylimipramine (25 mg/kg) 60 minutes prior to intrahypothalamic microinfusions (4.0 ul bilaterally) of either isotonic saline (control group) or 5, 7-dihydroxytryptamine (5, 7-DHT - 3.0 ug/ul) in the vicinity of the ventromedial nucleus (VMN).

Compared to controls, the 5, 7-DHT treated animals exhibited a stable long-lasting high fat diet contingent hyperphagia and obesity. An analysis of variations in food intake across 51 days of daily vaginal smearing (to determine estrous cycle stage) revealed that animals receiving 5, 7-DHT were significantly (t (19) = 4.00, $p < .01$) hyperphagic with respect to controls during the estrous stage of the cycle. However, during the diestrous stage of the cycle 5, 7-DHT animals were normophagic (t (19) = 1.63, $p > .05$). These data are taken to indicate that the serotonin depletion-induced hyperphagia reported here and in our earlier work is the result of depleted animals failing to show the typical "estrogen-induced-anorexia." Further work is needed, however, to evaluate the possibility that the so-called "estrogen-induced anorexia" is the result of estrogen activating a serotonergic circuit that is inhibitory with respect to feeding.

166.1 AN ETHOLOGICAL ANALYSIS OF MATING IN NAVANAX INERMIS (MOLLUSCA; OPISTHOBRANCHIA).

J.L. Leonard* and K. Lukowiak* (SPON: G. Mpitsos). Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 1N4.

As the first stage of a neuroethological study of sexual behavior in Navanax, we have developed an ethogram and described the events involved in courtship and copulation.

Navanax is a simultaneous hermaphrodite, which normally copulates in pairs with one animal assuming the male and the other the female sexual role. Copulations occur in bouts with the members of a pair exchanging sexual roles with each copulation. Bouts of as many as 6 copulations with strict alternation of sexual roles have been observed. The alternation of sexual roles is accompanied by changes between behaviorally identifiable "male" and "female" states.

At least eight different action patterns are involved in courtship and copulation in Navanax. Two action patterns are "female" and six "male". Courtship (= pre-copulatory) behavior is initiated by the male who turns onto and follows the mucous trail of a conspecific. After catching up with the "female", he explores her caudal lobes and posterior parapodia with his head. She responds by spreading her parapodia, exposing the common genital aperture. Copulation last an average of 42 min and is terminated after the "female" turns and begins to show male courtship behavior toward the "male".

The "female" action patterns are "Spread-tail Up" and "ALF's (anterior lateral folds of head) Pointed". Both are strongly influenced by "motivational" factors. The "male" and "female" behavioral states are not mutually exclusive. In animals which have been isolated for several days it is not uncommon for an individual to begin courtship of a conspecific and fail to achieve intromission because it has stopped exploring the partner's parapodia and assumed the female copulatory posture. Spread-tail up may be elicited by very gentle mechanical stimulation of the caudal lobes and posterior parapodia, and/or by exposure to conspecific mucous. Isolated animals often spend long periods of time with spread parapodia and ALF's Pointed.

Sexual behavior in Navanax is complex involving several action patterns which are influenced by both specific stimuli and by "motivational" factors related to to different behavioral states. This system should prove useful in understanding the physiological mechanisms underlying complex behavior.

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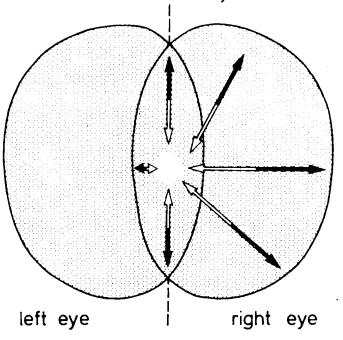
166.2 THE SEROTONERGIC METACEREBRAL CELLS MAY CONTROL BEHAVIORAL STATE IN PLEUROBRANCHAEA, R.A. Palovcik and J. L. Ram. Wayne State University, Detroit, Michigan 48202.

Serotonergic metacerebral cells (MCC's) have been presumed to specifically modulate feeding behavior in gastropods. An alternative function for the MCC's in Pleurobranchaea is that they mediate transitions from the still state to more alert states. Injection of intact animals with serotonin (10^{-7} moles/kg) enhanced behavioral state but never produced feeding. Comparison between recording from MCC's during different behavioral states were significant only when the still state was compared to other states (alert, moving, proboscis, or radula). Activation of the feeding motor program via stomatogastric nerve stimulation only moderately excited the MCC's. Highest rates of MCC firing were produced by aversive body wall nerve stimulation which suppressed the feeding rhythm. Since it has previously shown that feeding is more easily elicited under higher behavioral states (Lee and Palovcik 1976, Behav. Biol. 16, 251-266), MCC activation may subserve a more general arousal in Pleurobranchaea.

166.3 MOTION OF A FLOW-FIELD IS THE OPTIMAL STIMULUS FOR THE LANDING RESPONSE OF THE BLOWFLY, CALLIPHORA. H. Eckert University of Marburg, Zoology, P.O.Box 1929, D-3550 Marburg, FRG

The landing response (LR) of flies was elicited by moving grated patterns. The number of LR (upward throw of both foreleg tibiae) elicited by 20 stimulus repetitions was taken as response strength. The strength of the reaction depends sinusoidally on the direction of motion. The preference direction, (i.e. the direction of motion eliciting maximally strong responses), vary with the eye region stimulated: they are arranged radially with an origin in the direction of flight. If the influence of binocular interactions is eliminated (by painting one eye with black paint) then the preference directions follow the axes of the compound eye differing from the preference directions determined in 'binocular' flies. Only by binocular computation is the radial distribution of preference directions achieved: e.g. the vertical preference direction in the binocular region of the eye is based on horizontal (or oblique) preference directions determined in 'monocular' flies. Thus, nature has found a way to achieve changing preference directions (radial distribution) based on a homogeneous distribution of elementary movement detectors which are arranged along the horizontal axis of this compound eye. The figure shows the arrangement of preference directions (black arrows) together with inhibitory directions of movement (open arrows) in a scheme of the hexagonal facet array of both eyes. The central white patch denotes the direction of flight.



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166.4 CORRELATED CINEMATOGRAPHIC AND ELECTROMYOGRAPHIC ANALYSIS OF BEHAVIOR IN THE LAMPREY. Galen Eaholtz*, Joseph Ayers and Gail A. Carpenter. Marine Science Institute and Departments of Biology and Mathematics, Northeastern University, Boston, MA 02115.

We have developed a technique which allows direct comparison of behavior and electrophysiological recordings in freely behaving lampreys. Specimens are implanted with recording leads (0.001 in stainless steel) in one or several segmental myotomes and released into photographic trays for electromyographic recording. Both aquatic behaviors (swimming, burrowing, aversive withdrawal) can be intitiated in this environment, by applying appropriate releasers, as well as crawling by reducing the water level. A signal common to both an 8mm film and the electrophysiological recording is provided by a small lamp in the field of the motion picture. The lamp is illuminated by irregular pulses triggered by a hand held switch and the voltage powering the lamp is recorded on FM tape with the electromyograms. The shape of the lamprey's body and the status of the lamp (on or off) is digitized with a magnetic tablet for each frame of the motion picture. The digitized motion pictures are then analyzed on a frame-by-frame basis by computer algorithms (Soc. Neurosci. Abs. 6:466) which extract the locus (position) and curvature of axial flexions from the digitized images of the lamprey's body shape. A plot of the locus of axial flexions versus time (movie frame number) reveals linear conduction of flexion waves down the body. If the abscissa of the locus versus time plot is adjusted to the chart recording time base, the electromyographic recording (electrical activity at constant locus) can be directly related to the behavior by aligning light status pulses from the film with the lamp voltage voltage pulses recorded with the electromyogram.

We are currently applying this technique to determine the motor programs which underly different lamprey behaviors as well as to assess the neuronal basis of behavioral deficiencies (Soc. Neurosci. Abs. 7:681) observed in specimens recovering from complete transection of the spinal cord.

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166.5 SPINAL CONDITIONING: CIRCUIT ANALYSES. R.C. Durkovic. Dept. Physiol., Upstate Medical Center, Syracuse, NY 13210

In studies of a simplified neural model of learning, paired stimuli are delivered to the hind limb of the spinal cat in a classical conditioning paradigm. As a result flexion reflexes undergo changes that exhibit the characteristics of classically conditioned responses, the majority of such data being obtained by recording from ankle flexors [e.g. JCPP 84:88(1973), Physiol. Behav. 14:297 (1975)]. The present study was conducted:

(A) to compare simultaneous reflex alterations during conditioning in physiological flexor muscles of the knee [semitendinosis (ST)], ankle [tibialis anterior (TA)], and toes [extensor digitorum longus (EDL)], and

(B) to examine the changes in the Aα conditioned stimulus (CS) spinal circuitry of these muscles during the course of conditioned reflex facilitation induced by CS activation of Aα and Aδ cutaneous fibers.

The CS was electrical stimulation of the saphenous nerve (10/s for 1.5 s) at an intensity supramaximal for Aα and Aδ fibers. Random intertrial intervals averaged 3 min. Probe CS alone trials which activated Aα fibers but were subthreshold for Aδ fibers were delivered before and intermittently during acquisition. The unconditioned stimulus (US) was a 30/s, 0.5 sec train of stimuli to the superficial peroneal nerve at an intensity that activated all Aα and Aδ cutaneous fibers. For conditioning animals the US was delivered during the last 0.5 sec of the CS train. For sensitization control animals the US followed the CS by at least 30 sec. Flexion reflex responses to the CS and US were measured by tension transducers attached to the distal tendon of each muscle.

During conditioning reflex responses to the Aαδ CS increased rapidly in ST and TA compared to preacquisition levels. In control animals responses to the CS declined. In contrast, reflex changes in EDL were small and did not exhibit the patterns displayed by ST and TA. Nor did the US excite EDL as it did ST and TA.

During conditioning, probe Aα CS inputs indicated that the Aα circuitry is not usually involved in the conditioned reflex facilitation seen in ST and TA. These studies suggest that the spinal circuitry involved in spinal conditioning is specific for each motoneuron pool requiring excitatory inputs from both CS and US and involving pathways activated by Aδ cutaneous fibers.

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166.6 CLASSICAL CONDITIONING OF LYMNAEA STAGNALIS, T. Audesirk, J. Alexander*, G. Audesirk. Div. of Biol. Sci., Univ. of Missouri, Columbia, Mo. 65211.

The pond snail Lymnaea stagnalis is capable of rapid acquisition of a learned association between food (a sucrose-casein mixture: UCS) and a non-food chemostimulus (amyl acetate: CS). The training procedure consists of adding the CS to the water housing the snails, followed 15 sec later by the UCS. Snails then remain in the presence of both stimuli for the next two minutes. Fifteen CS - UCS pairings result in a significantly greater number of feeding movements to the CS by the experimental group than by their random controls. The elevated response persists for at least three weeks, and can be restored by brief (3 pairing) retraining. We found that both learning and expression of the learned response (rasping to the CS) are dependent on motivation which, in turn, is age-dependent.

Learning in Lymnaea shows several parallels with mammalian learning. For example, massed training is less effective than spaced training, especially when retention of the response is compared. Backward conditioning (presenting the CS 15 sec after the UCS) is less effective than forward conditioning, although both procedures result in learning. One striking finding is that the surroundings in which the snails were raised influenced their subsequent ability to be classically conditioned. Snails raised in "enriched" environments (including gravel, algae, and other snail species) acquire the conditioned response more readily than those raised under "impoverished" (bare tank) conditions, although both were fed the same high protein diet.

The neural circuitry underlying feeding in Lymnaea is known in some detail (e.g. Benjamin and Rose, TINS, 1980) making this gastropod a promising system for the study of neuronal changes underlying a learned modification of feeding.

166.7 NEURAL CORRELATES OF CONDITIONED CLAW CLOSING IN CRAYFISH. W. Stern-Tomlinson and G.L. Gerstein, Dept. Physiology, Univ. Pa. Sch. of Med., Philadelphia, PA 19104

In the following study, we examined various spike train parameters in order to determine their candidacy as neural codes for closing behavior. Crayfish were operantly conditioned to maintain the dactyl within a certain angular range, using a training paradigm involving an aversive stimulus (shock to the tail). The task was closing from a more open position. In some cases, appropriate tactile stimulation was given during the period of training. This period was followed by a test period in which claw position was monitored, in order to assess the duration of the trained behavior. It was determined from control data on the duration of tactile stimulus effects that, for the most part, tactile stimulation did not greatly affect test period performance.

The neural basis for closing behavior was then investigated by recording, during behavior, from proprioceptive (PD) neurons and from the synergist motor neurons slow closer excitor (SCE) and opener inhibitor (OI).

(1)Firing of PD neurons was sometimes periodic; this periodic driving of SCE and OI influenced firing of SCE and OI, but did not affect behavior in any obvious way. Therefore, such spike patterning is neither destructive of behavior, nor is it a relevant coding parameter.

(2)In different individuals closing behavior was achieved by alternate strategies, involving increases in the firing rate of SCE alone, or of both SCE and OI. Therefore, increased SCE firing appears obligatory. The behavioral significance of the alternate strategies is not known.

(3)SCE and OI have a greater than random probability of firing within about 100ms of one another. Quantitative changes in coincident firing did not correlate consistently with changes in dactyl position. However, the normalized amount of coincident firing increased with increase in SCE or OI firing rate.

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167.1 ELECTRIC ORGAN DISCHARGE PATTERNS IN THE SKATE (RAJIDAE) AND THEIR RELATION TO BEHAVIOR, B. Bratton* and J. Ayers (SPON: John Neumeyer). Biology Department and Marine Science Institute, Northeastern University, Nahant, Mass. 01908.

A quantitative analysis of the electric organ discharge (EOD) patterns in the Little Skate (Raja erinacea) and the Winter Skate (R. ocellata) was undertaken to characterize such properties as pulse duration, frequency, train duration, amplitude and pattern. These EOD properties were also correlated with the animal's behavior.

It was found that the skate's discharge has a characteristic pulse duration depending on the species under investigation. The pulse duration for R. erinacea averages 68 msec. while a sibling species, R. ocellata, has a pulse duration averaging 189 msec. Other properties described in this study have shown that R. erinacea has a characteristic discharge frequency of 2 to 7 pulses per second. The skate can discharge the electric organ at the low rate of 2 to 3 pulses per second for periods of a few seconds to several minutes.

The pulse amplitude varies in a way similar to the frequency. It ranges from a maximum of 1.5 volts to a minimum of 0.1 volt. This variation takes place during most of the discharge trains observed.

To test the idea that a skate will respond to the electrical discharge of another skate, the animals were subjected to artificially produced pulses from a stimulator. Experiments in which a stimulus is similar in pulse duration, amplitude and frequency, proved to be ineffective in evoking a response from the skates. The skate would discharge to a d.c. stimulus if the polarity was positive but would not respond to a negative stimulus at "make". Only when the negative stimulus was turned off would the skate discharge.

It was demonstrated that the releasing stimuli for discharge was physical contact with another skate. Skates isolated from other animals rarely discharge whereas groups of skates are found to discharge regularly. In some approach behaviors, two skates may respond to each other with interacting EOD displays.

The specific occurence of the EOD with ongoing behaviors suggests a possible role in communication. This proposed function for the EOD in Rajidae is based on the occurrence of the EOD when skates come into contact with each other and the lack of the EOD display during other individually isolated behaviors.

Supported by the Alfred P. Sloan Foundation.

167.2 SIGNIFICANCE OF ELECTRORECEPTOR TUNING IN THE RECOGNITION OF SPECIES SPECIFIC SIGNALS IN MORMYRID ELECTRIC FISH. C. D. Hopkins and A. H. Bass Section of Neurobiology and Behavior; Cornell University; Ithaca, New York. 14850.

Knollenorgan electroreceptors are the putative electric communication sensors of Mormyrid electric fishes. They are electrically tuned to frequencies most prevalent in the power spectrum of the species-specific electric organ discharge (EOD), which is spike like. Tuning is very broad (Q10's range from 0.1 to 0.9) to match the broad spectral bandwidth of the EOD.

Field work on the Mormyrids of the Ivindo River of Gabon (West Africa) has shown that there are two classes of Knollenorgan receptors in most species of Mormyrids. Low frequency organs are located on the lateral and ventral sides of the head, and respond most sensitively to stimuli between 500 and 1500 Hz; high frequency organs are located on the dorsal surface of the head, and over the rest of the body, and are tuned one octave higher.

Knollenorgans respond to natural stimuli (EODs from other individuals) by generating spikes which are phase locked to the EOD waveform. Knollenorgans respond to outside-negative to outside-positive going voltage transitions. Inversion of the stimulus polarity causes a shift to a different phase of the stimulus waveform.

Low frequency organs respond to gradual transitions in the waveform, preferentially, while high freqency receptors respond to more rapid transitions. When presented with a complex waveform like an EOD, low and high frequency receptors fire spikes at different, yet characteristic latencies. Each species-typical EOD from the field site evokes a unique temporal pattern of spikes.

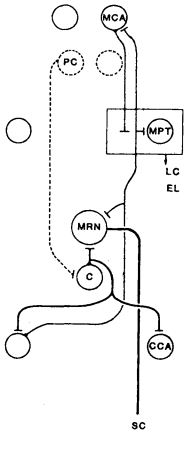
It is likely that the difference between low and high frequency receptors arose to increase the complexity of temporal sampling of species specific EODs, and hence reduce ambiguity in species recognition.

167.3 CENTRAL PATHWAYS OF THE ELECTRIC ORGAN DISCHARGE COMMAND IN MORMYRID FISH. C.Bell, S.Libouban*, T.Szabo*. C.N.R.S., Lab. Physiol. Nerv., Dept. 3, Gif-sur-Yvette, 91120, France.

The motoneurons to the mormyrid electric organ are driven by a descending volley from a medullary relay nucleus (MRN). The MRN does not itself initiate the electric organ discharge (EOD) but is driven by another center (Aljure, 1964). One goal of the present study was to identify this center anatomically. A second goal was to establish the pathways responsible for the corollary discharges of the EOD command in the electrosensory lobe (EL), where electroreceptors terminate.

The species Gnathonemus petersii was used. Electrical activity preceding the EOD was recorded extracellularly using pipettes filled with HRP in saline. After locating a structure with such activity, HRP was injected by iontophoresis.

A group of smaller cells is found immediately beneath the large cells of the MRN. The smaller cells are suggested as the origin of the EOD command and are thus provisionally referred to as the command nucleus (C). Axons of C terminate densely in MRN. Axons of C also project to a caudal bulbar nucleus, the caudal command associated nucleus (CCA). A mesencephalic nucleus, provisionally referred to as the pre command nucleus (PC),



appears to have a strong projection to C. The connection from PC to C is the only one in the diagram not yet confirmed with both retro- and anterograde transport. The axons of the cells in CCA ascend and cross the midline. They send collaterals to MRN and to the medial paratrigeminal nucleus (MPT), before terminating in a small mesencephalic command associated nucleus (MCA). The latter nucleus was studied by Aljure (1964) and Szabo (1979). The axons of the MCA descend to a region medial to MPT, where cells projecting to the electrosensory lobe (EL) are located. The EL may also be reached via MPT which projects to lobus caudalis (LC), a source of afferents to EL.

The cells of MRN appear to project only to the spinal cord (SC). The cells of C appear to project only to MRN and to CCA. Thus if C is indeed the origin of the EOD command, then the corollary discharges must be transmitted by way of the CCA.

167.4 HOW TO TALK TO A COCKROACH: BEHAVIORAL DISCRIMINATION AMONG ACOUSTICALLY DISTINCT SOUND SIGNALS IN *GROMPHADORHINA PORTENTOSA*. M.C. Nelson and R.K. Potter*. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

Close-range sound communication figures prominently in the courtship of *G.portentosa*, the giant Madagascar hissing cockroach. Several acoustical features distinguish courtship sounds from other social and non-social sound signals; the two features that most reliably sort sounds by context are relative amplitude or sound level (SL) and the temporal pattern of amplitude modulation (AM). (All sounds are broadband and frequency does not vary with context.) We carried out experiments to determine whether these features are behaviorally relevant to the recipients of sound signals, and if so, which features of courtship sound are important in promoting female receptivity and hence successful courtship.

The mating success of muted males, unable to vocalize, is only 1/10th that of vocalizing males; playback of recorded courtship hisses restores normal mating success (Nelson & Fraser, Behav Ecol & Sociobiol 6,'80). We compared disturbance hisses (high SL, abrupt onset) with courtship hisses (low SL, slow onset w/ rapid AM). By shifting the amplitude of recorded signals during playback we were able to compare the importance of AM vs SL features. Thus, animals in 4 playback groups heard one of 4 signals at the appropriate moments during courtship: CN, Courtship at Normal SL (62 dB); DN, Disturbance at Normal SL (80 dB); CH, Courtship at High SL (80 dB); or DL, Disturbance at Low SL (62 dB). Control groups could vocalize normally, or were muted but heard no playback signal. We scored female receptivity and mating success, and measured latency and frequency of several distinctive courtship displays for single males paired with trios of virgin females, to gauge the normality of interactions in different groups.

We conclude that females need both AM and SL cues for receptive behavior. 1) Courtship AM is not a sufficient cue: CH, like DN, is ineffective in eliciting receptivity (see Table). 2) Low SL is not a sufficient cue: DL is less effective than CN in eliciting receptivity. Only a signal that combines low SL and the appropriate AM pattern (i.e. CN) is adequate to elicit normal receptive behavior from females, who thus appear to be analysing the signals emitted by males in both the amplitude and temporal domains.

Group	courtship AM	courtship SL	♀recep-tivity	mating success
CN	yes	yes	+++	+++
DL	no	yes	+	++
CH	yes	no	-	---
DN	no	no	---	---

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167.5 INFERIOR LARYNGEAL NERVES CONTROL ULTRASONIC COURTSHIP VOCALIZATIONS IN MALE RODENTS. A. A. Nunez, S. M. Pomerantz and N. J. Bean*. Dept. of Psychol. & Neuroscience Program, Michigan State Univ., East Lansing, MI 48824.

Adult male rodents of *different species* produce ultrasounds as part of their precopulatory behavior. The temporal patterning and the frequency of the ultrasounds are species-specific; however in all cases they seem to facilitate copulations. In the present study we investigated the peripheral neural mechanisms involved in the production of ultrasounds in males of two species of myomorph rodents.

In house mice (Mus musculus) of the Swiss Webster strain, bilateral or unilateral cuts of the inferior laryngeal nerve (inferior cuts) abolished the production of 70 kHz ultrasounds in a large proportion of the animals (100% after bilateral cuts; 70% after unilateral cuts). Some recovery of function was evident *after* 2 weeks in animals with unilateral cuts. Bilateral cuts of the superior laryngeal nerve (superior cuts) or sham surgery did not reduce the proportion of animals that emitted 70 kHz ultrasounds during tests with adult females.

In deer mice (Peromyscus maniculatus), superior cuts or sham surgery did not prevent the production of 35 kHz ultrasounds during courtship behavior. On the other hand, only 14% of the males receiving unilateral inferior cuts produced ultrasounds on behavioral tests conducted 1 week after surgery. Males with unilateral inferior cuts recovered the ability to produce 35 kHz ultrasounds 3-4 weeks after surgery. This recovery of function was reversed by transections of the contralateral inferior laryngeal nerve.

The present results confirm that the inferior laryngeal nerves innervating the larynx are responsible for the motor output involved in ultrasound production. Previous work with rats has shown that the inferior laryngeal nerves originate in the nucleus ambiguus. Preliminary data from HRP studies suggest a similar pattern of laryngeal innervation in house mice and deer mice.

All the surgical procedures for the present study were done following the guidelines recommended by the NIH.

167.6 BILATERAL INTERACTIONS WITHIN THE VOCAL CONTROL PATHWAY OF BIRDS. K.R. Manogue*, J.A. Paton and F. Nottebohm (SPON: J. Cohen). The Rockefeller University, New York 10021.

Bilateral interactions between the right and left sides of the song control pathway of three avian species, the budgerigar (Melopsittacus undulatus), the zebra finch (Poephila guttata) and the canary (Serinus canarius) were compared using microstimulation and recording in anesthetized preparations. These birds were chosen in order to compare the physiological properties of each vocal control system with several anatomical and functional features known to differ among these species. Central and peripheral control of song in both oscine species is dominated by one side of the nervous system, although the lateralization in zebra finches is less marked than that of canaries. It is not yet known whether control of vocalization in budgerigars is centrally lateralized, but no peripheral laterality is present in a related parrot. The vocal control pathway of budgerigars has robust bilateral distributions both centrally and peripherally, while the canary (and we assume the zebra finch) have strictly ipsilateral connections between the principal efferent stations of the song control pathway. Stimulation of the highest of these efferent stations, the neostriatal nucleus hyperstriatum ventrale pars caudale (HVc), caused a constant-latency increase in neural activity of the tracheosyringeal (ts) nerves. These nerves supply the avian organ of voice, the syrinx. Unilateral stimulation of HVc in budgerigars caused equal responses in both right and left ts nerves (11-16 ms latency). In zebra finches and canaries, much larger responses were evoked in the ipsilateral ts nerve than in the contralateral ts nerve, as Arnold found when recording from syringeal muscle (Amer. Sci. (1980) 68, 165-173). The crossed responses in the oscines occured 3-5 ms later and at a higher threshold than the ipsilateral response. Also, the contralateral response could only be demonstrated with rates of stimulation exceeding 35/s. Simultaneous bilateral stimulation of HVc in budgerigars gives a greater than linear summation of responses on each ts nerve, evidence for convergence of ipsi- and contralateral inputs onto the same set of motor neurons. The bilateral distribution of excitation within the song control pathway of budgerigars should lead to coactivation of bilaterally paired muscles on each side of the syrinx. This type of interaction should ensure that the two sides of the parrot syrinx operate in a unitary, or single-voiced fashion. In contrast, the two sides of the song control pathway in the oscines are almost completely isolated from each other, and are thus free to act independently. This type of organization lends itself to the simultaneous production of unrelated sound elements by the two independent sound sources present in the songbird's two-voiced syrinx.

167.7 THE ROLE OF PERIPHERAL FEEDBACK IN THE MAINTENANCE OF VOCAL PATTERNS BY ZEBRA FINCHES. S. W. Bottjer & A. P. Arnold. Dept. of Psychology, UCLA, Los Angeles, CA 90024

Auditory feedback is essential for song learning in young zebra finches (Poephila guttata), but not for maintenance of song in adult birds (Price, '79, J. Comp. Physiol. Psychol., 93: 260). The latter result suggests that adult vocal behavior is either (a) dependent on feedback from the vocal organ (syrinx), or (b) not dependent on any source of peripheral feedback, but is instead controlled by a central motor program. We report preliminary results of an experiment designed to test the first possibility.

In zebra finches, afferent fibers originating in the syrinx leave via a branch of the hypoglossal nerve, and join the main trunk of the vagus to enter the brainstem dorsally (Bottjer & Arnold, '81, Soc. Neurosci. Abstr., 7: 270). It is possible to section hypoglossal afferent fibers, while leaving hypoglossal efferents intact, at the point where hypoglossal afferents join the vagus. The results presented herein represent our initial findings concerning the maintenance of stable song patterns in deafened adult zebra finches which have undergone section of either the left or right hypoglossal afferents. (Usually afferent and efferent fibers of the descending branch of the vagus are sectioned along with hypoglossal afferents; control experiments indicate that unilateral section of the descending vagus in deaf birds has no effect on vocal behavior.)

The song patterns of eight adult zebra finches were recorded; the birds then underwent bilateral removal of the cochleae and their song patterns were recorded again. Following this deafening operation, hypoglossal afferent fibers were sectioned on either the right (N = 4) or left (N = 4) side (hypoglossal efferents were left intact). Section of hypoglossal afferents on the left side disrupted the form of individual song notes in 1 out of 4 birds; section of afferents on the right side caused similar disruption in 2 out of 4 birds. (The overall tempo and structure of song were not affected in any case.) Interestingly, birds whose song was disrupted by unilateral section of afferent fibers tended to exhibit some minor changes in song as a result of deafening. All birds in which song was disrupted showed at least partial improvement as a function of time following the operation (8 to 19 days). This improvement was even observed in one additional bird following subsequent section of the intact, contralateral hypoglossal afferents. These preliminary results indicate that stereotyped vocal patterns produced by adult birds can persist in the absence of auditory feedback as well as feedback from the vocal organ.

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167.8 ANATOMICAL MAPPING OF THE CHICK MIDBRAIN CALL AREA: THREE-DIMENSIONAL THRESHOLD TOPOGRAPHY OF EVOKED VOCALIZATIONS. C. K. Bluhm* and R. E. Phillips. Lab. of Behavioral Physiology, 495 An Sci Vet Med Bldg., Univ. of Minnesota, St. Paul, MN 55108.

The primary objective of this study is to localize in greater detail the neural control areas of the avian midbrain which are most directly involved with electrically stimulated call production in anesthetized 1-3 day-old chicks (Gallus domesticus). After electrode placement, data collection, and histology were completed, we used computer-assisted data analysis to generate graphs of the three-dimensional threshold topography of evoked vocalizations for each planar section of the chick brain. These plots were then superimposed on an atlas of brain nuclei to delineate regions of the brain controlling low threshold calling.

The results of this study show that the chick midbrain contains a region localized in the nucleus intercollicularis (ICo) and nucleus mesencephalicus lateralis, pars ventralis (MLv) and linked to the archistriatum in the forebrain by the tractus occipito mesencephalicus (OM) pathway. The four midbrain call regions most important for low threshold calling are the (1) ICo, (2)MLv, (3) OM, and (4) the posterior commissure. Lowest threshold calls (10-30 μA) coincided with the anterior ICo and MLv, but the MLv was of greater importance as a continuous low threshold call area. Ventral to the ICo-MLv, a broad band of call sites occurs laterally. This band corresponds to MLv output fibers and is distributed caudally through the lateral reticular formation dorsal to the isthmic nuclei. This bundle exits the midbrain in a ventrolateral position and converges on the lateral pontine nucleus in the medulla. The thresholds for calling in the OM were always higher (40-60 μA) than those elicited from the MLv. The medial OM area and the lateral ICo-MLv call area were initially separate, but the two areas merged caudally. This suggests that the OM is an important input to the MLv call area.

Low thresholds for calling occurred throughout the anatomically diffuse MLv, but were lowest near the borders of the ICo and OM. The ICo, which has been previously implicated as the major midbrain call area, actually appears to have input going through it to the lowest threshold call area in the MLv. The ICo-MLv border had low threshold call sites, but the majority of these lay in the MLv.

In conclusion, the results of this study demonstrate that the control of call production in newly hatched chicks is similar to that of adult chickens. Moreover, our results demonstrate the applicability of computer graphics for use in analyzing large sets of neurophysiological data. By mapping this information on planar sections of a brain atlas, we can correlate neuroanatomical structures with specific behavior patterns.

167.9 RESPIRATORY GATING OF ACTIVITY IN THE AVIAN VOCAL CONTROL PATHWAY. J.A. Paton, K.R. Manogue*. The Rockefeller University, New York, NY 10021.

Vocalizations in birds are produced by air flowing past the membranes of the syrinx. The nature of the sounds produced depends on the characteristics of air flow and membrane position, which are controlled by the respiratory and vocal control systems of the central nervous system. In birds, the vocal control pathway contains a neostriatal nucleus, HVc; an archistriatal nucleus, RA; and a medullary motor nucleus, nXIIts, which innervates the muscles of the syrinx via the tracheosyringeal nerve.

To search for sites of interaction between the systems controlling respiration and vocalization, we electrically stimulated individual nuclei within the vocal control pathway and recorded respiratory phase and neural activity within the tracheosyringeal nerve. Experiments were done in budgerigars (Melopsittacus undulatus), zebra finches (Poephila guttata), or canaries (Serinus canaria) lightly anesthetized with Chloropent. Nuclei were stimulated with trains of 0.3 ms cathodal, constant current pulses (2-100ua) from 1 Mohm tungsten microelectrodes. Whole nerve responses were recorded from the ipsilateral tracheosyringeal nerve with a pair of hook electrodes covered with oil. Respiration was monitored with a thermistor placed in the path of tracheal air flow, such that the temperature of the thermistor increased during expiration.

In budgerigars, stimulation of HVc, RA, or nXIIts produced stimulus- locked potentials on the tracheosyringeal nerve. In each case stimulation recruited more activity during expiration than during inspiration, a phenomenon we refer to as respiratory gating. This gating seems to be a function of the excitability of the tracheosyringeal motor neurons, because the size of antidromically evoked potentials in nXIIts were also greater during expiration. The source and mechanism of this respiratory input to nXIIts remains to be determined. Respiratory gating was also found in zebra finches and canaries, both oscine songbirds.

As a result of respiratory gating, song related control of the syrinx is most effective during expiration when vocalizations normally occur. In effect then respiratory gating coordinates shared control of the same set of motor neurons during two different behaviors: breathing - an ongoing, automatic, medullary motor behavior - and singing - a complex, voluntary, learned behavior which is controlled by telencephalic nuclei.

Supported by NSF Grant BNS 79 24602, and USPHS Fellowship 1F32 MH08231.

167.10 ONTOGENY AND SEXUAL DIMORPHISM OF THE SONIC MOTOR NUCLEUS IN THE OYSTER TOADFISH. M. L. Fine, D. Economos*, J. R. McClung and R. Radtke*. Dept. of Biology and Physiology, Virginia Commonwealth Univ., Richmond, Va. 23284 and Pacific Gamefish Foundation, Honolulu, HI 96825.

Male oyster toadfish, Opsanus tau, produce a courtship boatwhistle call and have a larger sound-producing organ than females, who do not boatwhistle. Since number of neurons has been shown to increase postnatally in fishes, we considered ontogeny in our examination of the sonic motor nucleus (SMN) in the toadfish, a teleost known to live for about 12 years. Our purpose was to investigate the possibility of sexual dimorphism in ontogeny of the SMN. Fish were aged by counting annual rings on saccular otoliths and ranged from 1 to 11 years of age.

Compared to mammals in which brain growth is generally completed in early life, the toadfish brain retains a potential for increase in mass, cell number and cell size into later life. Brain weight increases for the life of the toadfish though this increase decelerates with increasing fish weight. Neuron number, ranging from 760 to 2888 in the SMN, increases rapidly to about 3 years, more slowly to about 8 years, and then levels off. There are no sexual differences in regressions of brain weight and SMN neuron number against fish size or age. Neuronal soma size in the SMN ranges from 9 to 35μm in average diameter and 67 to 916μm^2 in area. This increase in size can be observed for at least 7 years. Males have larger neurons than females. However, males can be separated into populations with large and small soma sizes. Neuron size is not different between females and males with small somas. Neurons in males with large somas are significantly larger than neurons in females and neurons in males with small somas ($p < .0001$). Such male dimorphism is reminiscent of other behavioral and morphological dimorphisms, which have led to characterization of males into territorial and satellite forms in certain mating systems.

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167.11 THE SEXUAL DIFFERENTIATION OF SOCIAL PLAY IN NORWAY RAT PUPS IS MEDIATED BY THE ANDROGEN RECEPTOR SYSTEM. M.J. Meaney*, J. Stewart, M.Y. McGinnis* and B.S. McEwen (SPON: Philip A. Femano). The Rockefeller University, New York, NY and Dept. of Psychology, Concordia University, Montreal, Canada.

Social play among juvenile Norway rats is sexually dimorphic; males engage in more play fighting than do females. The play fighting of juvenile females can be masculinized by neonatal exposure to either testosterone (T) or 5α-dihydrotestosterone, but not by estradiol. The neonatal castration of males results in female levels of play-fighting (Meaney and Stewart, Horm. Behav. 15: 197, 1981). Previous work (Goy, Recent Adv. Primatology, 1: 449, 1978) has shown that 5α- dihydrotestosterone, administered prenatally, also masculinizes social play in female rhesus monkeys. These findings suggest that in the Norway rat and the rhesus monkey androgens, interacting with the androgen receptor exert an organizational influence on social play.

We have further examined this hypothesis by studying the play-fighting of juvenile Tfm rat pups. Rats bearing the X-linked Tfm mutation are known to be insensitive to androgens and show a marked deficiency of androgen receptors. We found that prepubertal Tfm males play-fought no more frequently than did female pups, and that both groups engaged in significantly less play fighting than did normal male pups. In a second study, we found that male pups treated with the anti-androgen Flutamide (in Silastic capsules) during the neonatal period (days 1 to 10 of life) engaged in less play-fighting than did control males. Moreover, Flutamide-treated males, like the Tfm males, engaged in play-fighting at rates that were statistically indistinguishable from those of female pups.

In subsequent work we have been examining the androgen receptor system in the limbic brain of neonatal rats using a nuclear exchange assay and (^{3}H)R1881 as a ligand (Soc. Neurosci. Abst., this meeting). In one such study, we found that a Flutamide treatment that was comparable to that used to block the masculinization of social play dramatically reduced the nuclear translocation of the androgen receptor in various brain regions of T-primed pups. Taken together with previous data, the findings strongly suggest that the sexual differentiation of social play in juvenile rats is mediated by the activation of the androgen receptor during the neonatal period. In considering this finding it is important to note that social play is sexually dimorphic in a broad range of species and that is especially so in primates, including humans.

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167.12 CHEMOSENSORY CONTROL OF SPAWNING MECHANISMS IN GOLDFISH. Leo S. Demski, Joseph G. Dulka*, School of Biological Sciences, Univ. of Kentucky, Lexington, KY and R. Glenn Northcutt, Division of Biological Sciences, University of Michigan, Ann Arbor, MI.

Sperm release (SR) has been evoked in immobilized, lightly anesthetized goldfish by electrical stimulation of the olfactory tract (OT) using both suction and metal-hook electrodes. Thresholds were below 20 μA in 5 animals. Cutting or applying xylocaine to the OT proximal to the electrode blocked the responses, the latter being reversible. Responses can still be evoked with the contralateral OT severed and the ipsilateral lateral bundle of the OT (LOT) cut. Indeed, stimulation of the medial bundle (MOT) alone produces low threshold responses. The results suggest that chemosensory information carried in the MOT may influence SR mechanisms and perhaps other sexual motor pathways. Three functional systems are represented in the teleost OT: 1) afferents from the cells in the olfactory bulb; 2) fibers originating from neurons in the brain and ending on cells in the olfactory bulbs and 3) fibers of ganglion cells located on the surface of the olfactory nerves i.e. the terminal nerve (TN). Initially, we assumed that the SR resulted from either othodromic activation of afferent fibers and consequent excitation of their central targets or, less likely, antidromic activation of the efferent OT fibers and subsequent excitation of central areas via activated collaterals. However, recent studies suggest that activation of the TN system may be the most likely cause of the SR responses. Münz et al. (Cell and Tis. Res. 222:313) have demonstrated that the TN in sunfish projects to forebrain regions related to SR as well as the retina and that the system contains LHRH. Northcutt (unpublished) has demonstrated similar anatomical connections for the TN of goldfish, including evidence that dendrites of the cells extend into the olfactory epithelium. In goldfish TN fibers are located only in the MOT. To test the liklihood that the TN modulates SR in goldfish, we carried out the following experiments. Stimulation of the optic nerve at currents below 50 μA resulted in evoked SR. Control experiments (as above) and stimulation of other nearby nerves indicate that current spread to other structures was not involved. We theorize that the SR is triggered by antidromic activation of the TN fibers efferent to the retina with subsequent excitation of central SR areas via excited collaterals. Preliminary observations indicate that μl quantities of LHRH injected into the ventricle near the preoptic area (100 μg/ml) can trigger sustained and powerful SR activity. The pharmacological specificity of the response is yet to be determined. At this point, our data strongly suggest that activation of TN fibers in the MOT is responsible for triggering SR.

167.13 CENTRAL CONTROL OF EATING IN THE PIGEON: A SENSORIMOTOR CHAIN FROM GRAIN THROUGH BRAIN. J.M.Wild and H.P.Zeigler, Dept. of Behavioural Biology, R.S.B.S., Australian National Univ. and Biopsychology Program, Hunter College, City Univ. of New York.

The neural control of feeding in the pigeon involves contributions from afferent and efferent components of the avian trigeminal system. We have now identified structures which link the trigeminal sensory and motor systems, completing a sensorimotor circuit mediating grasping, pecking and feeding in the pigeon.

Somatosensory input from the oral region is conveyed to the telencephalon of birds by a group of trigeminal lemniscal ("quinto frontal") structures, including the principal trigeminal sensory nucleus (PrV), the quintofrontal tract (QFT) and its terminal field, the nucleus basalis (NB) of the telencephalon. Efferent pathways for oromotor activity include the cranial nerve motor nuclei of the trigeminal and facial nerves (V/VII) and premotor neurons located in the lateral (parvocellular) reticular formation (Rpc). These premotor neurons receive a descending projection from the telencephalon via the occipitomesencephalic tract (OMT), which originates in the anterior and intermediate archistriatum (Arch).

To complete a trigeminal sensorimotor circuit connecting the afferent and efferent components of the pigeon's feeding system it is necessary to identify the structure(s) linking NB with Arch. We have used a combination of orthograde and retrograde markers to identify the projection of NB and to determine the connections between its terminal field and that portion of Arch from which OMT originates.

Injections of ^{3}H proline into NB labeled a terminal field in the neostriatum caudale, internal to the ventricle and dorsal to Arch, within a region designated by Karten as the regio trigeminalis telencephali (rTT). Injections of HRP into this region retrogradely labeled cells in NB. Injections of HRP or tritiated amino acids into rTT produced dense labeling of a spherical terminal field within Arch. HRP injections into this Arch region retrogradely labeled cells within rTT, as well as anterogradely labeling OMT, which has previously been shown to project upon Rpc. We have previously demonstrated that HRP injections into this reticular region retrogradely label cells within the anterior and intermediate Arch and produce anterograde labeling of V/VII.

Our experiments demonstrate that these intratelencephalic structures (rTT, Arch) link the trigeminal sensory and motor systems in the pigeon. The identification of a trigeminal sensory telencephalic-trigeminal motor circuit adds feeding to the limited number of *vertebrate* behaviors for which such circuits are being delineated. (Supported by Grants BNS #791438 (NSF), MH-08366, MH-00320 (NIMH) and Biopsychology Program, Hunter College).

167.14 GRASPING IN THE PIGEON: FINAL COMMON PATH MECHANISMS. Bradley G. Klein and H. Philip Zeigler, Biopsychology Program, Hunter College, CUNY, N.Y.,N.Y. 10021 and Dept. of Ornithology, American Museum of Natural History, N.Y.,N.Y. 10024

Considered as an effector organ, the beak of birds functions in pecking, grasping and manipulation analogously to the hand of primates. Behavioral studies of pecking have shown that grasping involves an adjustment of gape size which takes place during head descent, is linearly related to seed size and is completed prior to tactual contact of the beak with the seed. We have used photographic, surgical & behavioral procedures to provide a quantitative description of the pigeon's grasping response, identify the motoneurons mediating this response and clarify their contribution to the control of grasping.

The topography of pecking & grasping were examined using high speed (400 fps) cinematography. A single frame camera,triggered by the pecking response, provided data on gape size at the point of maximal opening prior to grasping. A variety of seed sizes were used to derive gape size functions. The motor nerves to beak opener muscles were identified by dissection and their contribution to the control of gape was examined using a 2 stage denervation procedure. In the first stage the facial (VII) nerve branches innervating the opener muscle of the lower beak (M. depressor mandibulae) were sectioned. In the second stage section of the trigeminal (V) innervation of the opener muscle of the upper beak (M. protractor quadrati) was added. The effect of these procedures upon grasping topography was compared with those produced by sham surgery.

Behavioral studies show that production of gape involves both opener muscles. Smaller gapes involve only the upper beak (maxilla). Larger gape sizes are produced by jointly activating both beak openers. Section of N. depressor mandibulae abolishes opening of the mandible & produced a significant reduction in gape. Subsequent section of N. protractor quadrati removes the contribution of the maxilla & no significant gape was evident.

These data indicate that N. depressor mandibulae & N. protractor quadrati constitute the final common path for beak opening during grasping. The cell bodies of these motoneurons lie in the V/VII motor nuclear complex and our previous HRP studies show that they receive monosynaptic inputs from sensory (proprioceptive) neurons in N. Mesencephalic V & interneurons in the Lateral Reticular Formation & disynaptic inputs from the telencephalon. The identification of final common path neurons for opening-grasping will facilitate the use of this response as a neurobehavioral marker for studies of sensorimotor and motivational processes.

167.15 EVOLUTION OF FLIGHTLESSNESS: NEUROBEHAVIORAL EFFECTS. R. R. Provine. Dept. of Psych., Univ. Md. Baltimore Co., Catonsville, MD 21228.

The consequences of the evolution of flightlessness on the neurobehavioral process of the wing-motor system were evaluated by searching for drop-evoked and spontaneous wing-flapping in flightless emus and penguins. The commun emu (Dromaius novaehollandiae) was selected because its tiny wings provide no flight related function. Emus evolved from flighted forms by the Pleistocene. Humboldt (Spheniscus humboldti) and Black-footed (Spheniscus demersus) penguins were also tested. Extant penguins are flightless in air but evolved from flying ancestors as early as the Eocene. Although penguins lost their capacity for aerial flight, they are masterful flyers through the denser medium of water. Their drop-evoked wing-flapping performance was compared with that of the common puffin (Fratercula artica) and murre (Uria aalge) that are capable of both submarine and aerial flight. The results of the comparative analysis should indicate if a behavior and its neural mechanisms are conserved over millions of years of evolutionary history when they serve no apparent function. The present comparative analysis of the effect of flightlessness on emus and penguins over millenia contrasts with the typical deprivation experiment that examines the effect of a short interval of movement restriction during the lifetime of the individual (Provine, R.R., Behav. Neur. Biol., 27:233, 1979; Dev. Psychobiol., 14:279 and 481, 1981).

Emus performed neither drop-evoked nor spontaneous wing-flapping. Penguins produced no drop-evoked wing-flapping but performed powerful wing-movement during submarine "flight." In contrast, puffins and murres that are capable of both aerial and submarine flight performed spontaneous and drop-evoked wing-flapping. Drop-evoked and spontaneous wing-flapping of the emu and drop-evoked wing-flapping of the penguin were lost during evolutionary history.

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167.16 MOTILITY PATTERNS IN THE OPOSSUM DURING DEVELOPMENT. Richard W. Bunch, C.H. Narayanan, and Y. Narayanan*. Dept. of Anat., LSU Sch. of Med., New Orleans, LA 70119-2799.

The relationship of early fetal behavior and progressive differentiation of the developing nervous system has been under investigation in our laboratory. In this study, both quantitative and qualitative recordings of motility in the opossum were made beginning with prenatal stages and followed through to 42 days postnatally. Recordings of time spent in activity as well as responses to cutaneous stimulation were conducted with emphasis on changes of motility patterns with age. Wild opossums were bred in captivity. Laparotomies were performed on pregnant opossums and the uterine horns placed in Tyrode solution (35^{o}C, pH 7.0). The embryos were exposed within a specially constructed observation chamber (Bunch, et al., 1980) for subsequent observation and recordings of behavior. Pouch young were similarly exposed while inside the pouch. Activity was recorded with an Esterline Angus polygraph. Behavior was categorized into local (eg. isolated forelimb activity), partial (eg. forelimb and head), and total (eg. head, forelimb and trunk) motility patterns. Responses to cutaneous stimulation were recorded using a pressure graduated esthesiometer. Results of this study indicate that spontaneous motility begins at 12½ days post conception in the form of sudden bursts of activity comprised of head extension and forelimb abduction. Within 24 hrs, this motility transforms into a total pattern of integrated activity involving crawling movements of the forelimbs and synchronous rotation of the head and trunk in a cephalocaudal sequence. The total percent activity increased steadily throughout the observation period. Total activity is the most prevalent type of behavior until approximately 24 days after birth. Partial activity comes into the picture and shows an increase in frequency from here on. Partial and local motility patterns begin to increase after 32 days postnatally while total motility patterns show a decrease. Responses to cutaneous stimulation appear first in the perioral region at birth and in the forepaws 10-12 days later. These responses are at first of the total pattern type and later become more localized in nature. All 3 categories of motility were observed at every stage of development. By birth, the opossum has formed a predictable motility pattern in striking contrast to motility patterns of the chick or rat fetus at comparable stages of development. The changes in frequencies of the different categories of activity in the developing opossum may reflect different degrees of summation or recruitment of neuronal activity.

Supported in part by Biomedical Research Support Grant to the LSU Sch. of Dentistry to R.W. Bunch and HD-12064 from NIH to C.H. Narayanan.

168.1 PRENATAL AND POSTNATAL UNDERNUTRITION AND THE EFFECTS OF BRAIN DAMAGE. Marc D. Schrode*, C. Robert Almli, Stanley Finger, and Peter J. Morgane. Washington University School of Medicine, St. Louis, Mo. 63110 and Worcester Foundation for Experimental Biology, Shrewsbury, Ma. 01545.

The topic of in utero undernutrition has only recently begun to receive research attention. In utero undernutrition may be one of the major factors contributing to premature birth, and thus, small-for-gestation-age and small-for-date infants. Postnatal undernutrition in rats results in retarded (or altered) body weight, eye-opening, behavioral development, and nervous system development. Prenatal undernutrition might be expected to have similar or more severe effects. The present study is the first program determining the relations between pre- and postnatal undernutrition, neural development, and the effects of brain damage sustained early in life. Research has shown that lateral hypothalamic (LH) destruction sustained by rats younger than 6 days of age is associated with continued growth and life, whereas LH destruction sustained by rats 6 days of age and older is associated with body weight loss and death. The present study determines if undernutrition alters the time-course of LH-lesion-induced deficits in developing rats.

Prior to breeding, during gestation, and postnatally, dams and litters were maintained on normal (25% casein) or low-protein (6% casein) isocaloric (4.3 kcal/g) diets. The 6% and 25% pups sustained LH destruction at 10 days of age. At this age, 6% pups had body weights that approximated those of the 25% pups at birth.

Following LH destruction at 10 days of age, 25% rats ceased suckling, lost body weight, and died by 5 days postlesion. The 6% rats also ceased suckling, lost body weight, and they died by 3 days postlesion. Another difference was that the 6% rats died in response to smaller and less-well placed damage. However, 6% and 25% rats were similar for % daily body weight lost (from postlesion), % body weight lost at death (from prelesion), and % of control body weight through the day of death.

Undernutrition, and the potential for delayed neural development, did not protect the 6% rats from the effects of LH damage. In fact, the undernutrition may have exacerbated the brain damage effects. Brain damage (e.g. intraventricular hemorrhage) so common to premature human infants may be more devastating due to pre- and postnatal nutritional status. (Supported by BSRG grant S07RR05389 and NICHD grant HD06364)

168.2 EFFECTS OF FOOD RESTRICTION DURING LACTATION ON POSTNATAL DEVELOPMENT OF RAT CEREBELLAR CORTEX. A. Feria-Velasco, A.R. del Angel* and G. Tapia-Arizmendi*. Div. Developmental Biology. Unidad de Investigación Biomed. Occidente. I.M.S.S. Guadalajara, Jal. MEXICO.

Malnutrition at various stages of development has been reported to bring about a wide variety of changes in the central nervous system. In the present work, the effects of food restriction during lactation on the postnatal development of cerebellar cortex were studied in 169 male and female rats. A method described by Wiggins was used to impose food restriction during lactation. Four pups of each offspring of 8 were separated from their mothers 1 hr the day of birth, 2 hours the 2nd day, 3 hours the 3rd, and one more hour consecutively per day until the pups were separated 12 hrs the 12th day. The following days until weaning (21st day) the pups were separated from their mothers for 8 hrs per day, and after weaning the food was taken out the cages for 8 hrs at night and determinations were done at birth, 5, 10, 15, 20, 25 and 30th days in both, the control and experimental groups. Weights of body and cerebellum were recorded, and DNA, RNA and proteins were measured. Representative animals from each group were fixed by intracardial perfusion and sections from vermis and cerebellar hemisphere were postfixed and embedded in Epon 812. On 1 μm-thick sections, thickness of cerebellar cortex, external granular layer and molecular layer were measured and recorded.

Body and cerebellar weights showed statistically significant differences in the restricted animals in the order of 30-40%, and 16%, respectively. Cerebellar DNA and RNA contents in the restricted groups were 30% lower than in the normal animals at 5 and 10 days, and 12-18% at 30 days of age. A significant delay in disappearance of the external granular layer of cerebellar cortex was observed in the restricted pups, being more conspicuous in vermis than in cerebellar hemisphere. No significant differences were found in thickness of molecular layer when both groups of animals were compared and similar results were obtained in the total thickness of cerebellar cortex.

168.3 AMPHETAMINE ISOMERS FAIL TO DECREASE HYPERACTIVITY IN THE NEONATAL 6-HYDROXYDOPAMINE MODEL. J.T. Concannon and M.D. Schechter, Program in Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

In an attempt to replicate the findings of Shaywitz et al. (Nature 261:153, 1976) reporting that amphetamine decreases hyperactivity in developing 6-hydroxydopamine (6-OHDA)-treated rats, we utilized intracisternal injections of 6-OHDA or its vehicle, and measured activity during development using a time-sampling procedure. At 5 days of age, rat pups were pretreated with intraperitoneal desmethylimipramine and then were randomly assigned to either 6-OHDA or vehicle treatment using a littermate control design. Behavioral activity was measured at 15, 20, 25 and 30 days of age, using a repeated-measures design, after an intraperitoneal injection of either saline or graded doses of either d-amphetamine (0.125, 0.25, 0.50, 1.00 or 2.00 mg/kg) or l-amphetamine (0.25, 0.50, 1.00, 2.00 or 4.00 mg/kg). In vehicle and 6-OHDA-treated pups injected with saline, activity increased from moderately low levels at 15 days of age to moderately high levels at 25 days of age. Furthermore, 6-OHDA-treated pups injected with saline were hyperactive at 20 days of age. At 25 days of age, activity in both saline-injected groups was equal and, thereafter, declined to levels typical for adults. Administration of increasing doses of the amphetamine isomers generally increased activity in both vehicle- and 6-OHDA-treated rats at all ages and d-amphetamine was more potent than l-amphetamine in this respect. Perhaps most importantly, no dose of either amphetamine isomer decreased activity at 20 days of age in the 6-OHDA-treated hyperactive pups. Whole-brain dopamine (DA), assayed at 35 days of age, was significantly depleted (56%), whereas whole-brain norepinephrine (NE) was not significantly depleted (9%). Furthermore, the 6-OHDA treatment did not lead to decreased body weight at a time when hyperactivity was detected. Hence, no convincing evidence was found for a "paradoxical calming" effect of amphetamine in DA-depleted hyperactive rat pups, confirming the findings of other recent reports (Thieme et al., Psychopharmacology, 67: 165, 1980; Pappas et al., Psychopharmacology, 70:41, 1980). Since clinically-useful drugs do not reduce hyperactivity in this model, these results suggest that the neonatal 6-OHDA-treated rat does not appear to represent an accurate model for the pre-clinical investigation of human hyperkinesis.
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168.4 NEONATAL FEBRILE SEIZURES ALTER ADULT HIPPOCAMPUS MORPHOLOGY AND BENZODIAZEPINE BINDING. J. E. Franck,* J. Chisholm,* and C. K. Kellogg. Dept. Neurosurgery, Univ. of Wash., Seattle, WA (JEF), Dept. of Pharmacology and Dept. of Psychology, Univ. of Rochester, Rochester, NY.

Febrile seizures in children are common and are thought to increase the risk of adult partial seizures or temporal lobe epilepsy. Despite this association there is a puacity of experimental work on the neurologic sequelae of this type of convulsion. We have found that a single neonatal febrile seizure (NFS) induces selective morphologic and benzodiazepine (BDZ) binding changes in the hippocampus of adult rats.

A febrile seizure was induced on postnatal day 15. Control pups were removed from the dam for a comparable time period. At twenty days of age there was a proliferation of reactive astrocytes restricted to the hippocampus (Cajal and Holzer methods). This reaction disappeared by 60 days. At 20 and 60 days hippocampal cells appear normal in Nissl stained material. There was no evidence of ongoing neuronal degeneration at any age studied (Fink-Heimer method). By 90 days, however, neurons in the CA3 and CA2 regions of the hippocampus were grossly shrunken and basophilic and were bounded by an increased extraneuronal space. These observations suggest a neuronal loss of water, with condensation of the cytoplasm. This reaction was not seen in other subfields nor was it seen in other brain regions or in control animals.

The BDZ binding site and its putative endogenous ligand may play a role in seizures, hence BDZ binding was measured as an initial attempt to characterize the nature of the hippocampal alterations following NFS. Binding was determined by displacement analysis of ^{3}H flunitrazepam with CL 218-872, a non-BDZ anti-seizure agent which, unlike the BDZs, displays high affinity for a specific subpopulation of BDZ sites. A NFS produced a significant decrease in the IC_{50} (418 $\pm$ 28 nM vs 624 $\pm$ 62 nM) and the Hill coefficient (.570 $\pm$ .018 vs 694 $\pm$ .041) in the hippocampus at 90 days but not at earlier ages. Cortex and cerebellum were unchanged. The Bmax remained constant following NFS indicating that the number of binding sites is unchanged but a greater proportion are of the high affinity type. An increase in high affinity BDZ sites in the hippocampus following NFS suggests that the morphologic changes have functional implications.

A single NFS produces changes specific to hippocampus. The subfields involved are similar to those most sensitive to epileptic insult in humans and which have been shown to be involved in seizure genesis in hippocampus in experimental work. The present data indicate that febrile seizures during development are not a benign event and require intensive study.

168.5 SENSORI-NEURAL DEGENERATION IN THE COCHLEA OF YOUNG (DEAF) SHAKER-1 MUTANT MICE. A. Shnerson*, R. Pujol*, T. R. Van De Water* and M. Lenoir* (SPON: R. D. Ginzberg). Lab. Develop. Otobiology, Albert Einstein College of Medicine, New York, New York 10461 and Laboratoire de Neurobiologie de l'Audition, Montpellier, France.

The study of mutant mice with genetic anomalies of the inner ear can provide insights into the processes which act to form a normal inner ear. Further definition of the nature and course of postnatal changes in the sensory and neural components of the inner ears of shaker-1 mice was, therefore, undertaken. The cochleas of 3-, 6-, 10-, 12-, 18-, and 30-day-old mice, homozygous for the shaker-1 gene (sh-1/sh-1), were examined using transmission electron microscopy. Relative to observations in the normal mouse (Shnerson, A., Devigne, C. and Pujol, R., Dev. Brain Res., 2:77, 1982), the present findings indicate retarded development coupled with progressive degeneration of early onset in Corti's organ, its nerve supply, and the cells of the spiral ganglion. Especially noteworthy are the following: Both outer hair cells and spiral ganglion cells of 3-day-old mice were already abnormal. Spiral ganglion neurons were especially loosely ensheathed by glial cells and were in direct contact with nerve fibers. Outer hair cells contained vacuoles and lysosomes. By 6 days of age inner hair cells were similarly affected. Most of the afferent nerve supply of the organ of Corti had degenerated by Day 18. The behavior of efferents within Corti's organ was complex. Efferents arrived late (Day 12) at the outer hair cells, were few in number, formed only immature synapses with these cells, and subsequently degenerated. In contrast, the efferent nerve supply of the inner hair cells appeared normal.

The selective degeneration of efferents to outer hair cells is viewed as being consistent with the hypothesis that there are two, independent, efferent systems which innervate the two types of cochlear sensory hair cells. The simultaneous occurrence of organ of Corti and spiral ganglion cell anomalies suggests that the shaker-1 mouse may be a useful model animal through which to gain insights into the role of sensori-neural interactions in the phenotypic expression of genetic inner ear defects.

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168.6 HYPOMYELINATION AND RECOVERY OF THE MYELIN DEFICIT IN FEMALES HETEROZYGOUS FOR THE JIMPY GENE. J. Rosenfeld, V.L. Friedrich Jr. Laboratory of Neuromorphology, Univ. of Connecticut U-154, Storrs, Connecticut 06268.

Jimpy is a sex linked murine mutation producing a severe hypomyelination throughout the central nervous system in affected hemizygous males. Consistent with Lyon's hypothesis, the effect of X chromosome inactivation has been observed in females heterozygous for the jimpy gene. The result of such X chromosome inactivation is a mosaic pattern of myelin distribution and gliosis, observed thus far only in the optic nerve (Skoff et al.). The process of X chromosome inactivation should, however, affect all brain regions. We wondered whether the amount of myelin in the heterozygote might be reduced in regions which do not exhibit visible 'patching' of myelin.

In this study we used point hit counting of electron micrographs from the anterior commisure to determine the amount of myelin in females heterozygous for jimpy ($X^{Jp}X^{+}$) and in controls ($X^{+}X^{+}$) at 43 and 201 days after birth. A 20% decrease in the amount of myelin was observed in the younger jimpy heterozygote compared to the same age control.

We found no difference in myelin content between the older jimpy heterozygote and its control. In control animals the amount of myelin was the same at the two ages. By contrast, the amount of myelin in the older heterozygote was increased substantially over the value for the young heterozygote.

In the light microscope, we could find no evidence of unmyelinated patches in the anterior commisure or in other fiber tracts of the brain and spinal cord. However, a similar reduction in the amount of myelin at the young age and a subsequent increase at the older age may be occuring in other areas. We observed an increase in myelin content of the jimpy heterozygote with age. This may reflect a compensatory recovery of myelination. The jimpy heterozygote might be a suitable model for the study of the regulation of myelination and remyelination in the central nervous system. We are presently expanding the study to include other brain regions and younger animals.

168.7 HYPOXIA AND PYRAMIDAL CELL SPINE FORMATION AND DENDRITIC ARBORIZATION: A QUANTITATIVE GOLGI STUDY. M.C. Yu*; D. Spero* and F. Hackett*. (SPON: N. Ingoglia). Dept. of Anatomy, New Jersey Medical School, Newark, New Jersey 07103.

A study was made to determine the effect of hypoxia on the development of dendritic spine and dendrite arborization of the pyramidal cells of the rat parietal cerebral cortex. New-born Sprague-Dawley rats were culled into 8 pups per dam. On postnatal day 5, they were placed into an hypoxic chamber and exposed to a low oxygen environment (10% O_2; 90% N_2) for 7 consecutive days. During the period of hypoxia, the pups were removed from the chamber twice daily at 9:00 AM and 4:00 PM for supplemental feedings with an enriched liquid diet, in order to minimize the factor of undernutrition as a result of possible decreased milk production by the dams. For control, another group of rats, also culled to 8 pups per litter, were exposed to normal ambient air and nursed by their dams. On postnatal day 12, one week after the hypoxic exposure, all the rats were taken out of the chamber. One group of rats was killed immediately while the other groups were cross-fostered by other, normal lactating dams, and killed at postnatal days 17, 22, 40 and 80, respectively. The rats were perfused intracardially with 10% phosphate-buffered formalin. The brain was removed to be processed by the rapid Golgi method, and serial sections were cut at 80 microns. Dendritic spines on the apical dendrite of pyramidal cells at Layers III and V were assessed quantitatively at successive increments of 50, 100, 150 and 200 um from the cell body. Apical and basal dendrites were also enumerated at similar intervals of distance from the cell body. At day 12, the number of spines of the control group was consistently greater than the hypoxic group. At day 17, however, the number of spines of the hypoxic group was found to slightly exceed the control. Thereafter, the spines of the hypoxic group were equal to the control at postnatal days 22, 40 and 80 respectively. These data suggested that spine formation was retarded during the period of hypoxia, but substantial recovery occurred after the removal of the hypoxic stress. The alteration in spine number will be correlated with the altered pattern of dendritic arborization.

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168.8 PRENATAL L-DOPA TREATMENT DIFFERENTIALLY AFFECTS ADULT COPULATORY BEHAVIOR. C. Marschall and L.G. Clemens. Michigan State University, East Lansing, MI. 48824

Dopaminergic neurotransmission in the brain may facilitate masculine copulatory behavior in adult male rats. Sexual behavior in castrated (Malmnas, Pharmacol.Biochem. Behav.4:521, 1976) or sexually sluggish males (Tagliamonte,et al, Pharmacol. Biochem. Behav.2:257, 1974) is increased following systemic treatment with L-DOPA. The role of CNS dopamine in the mediation of feminine sexual behavior is not clear.

Maturation of the dopaminergic system in the CNS begins early in ontogeny (Coyle and Henry, J. Neurochem 21:61, 1974). Thus the prenatal period is a particularly sensitive period in which manipulation of neurotransmitter levels may alter normal development with possible long-term effects on function.

Pregnant Long-Evans rats were injected i.p. with L-DOPA (30 or 60 mg/kg saline) daily on prenatal days 10-21. Controls received saline alone. At birth litters were adjusted to 5 males, 5 females. Animals were weaned on day 25 postnatally. Female offspring were ovariectomized between 60-70 days of age and tested first for the display of lordotic behavior following estrogen-progesterone priming.Masculine copulatory behavior was assessed weekly following treatment with 250 ug testosterone propionate daily. Male offspring were castrated and tested for the display of copulatory behavior following treatment with 100 ug testosterone propionate daily. The potential to display feminine copulatory behavior was tested following estrogen-progesterone priming.

Body weights of offspring at weaning did not differ among the treatment groups. Mount frequency was significantly elevated by the 4th week of testing in L-DOPA treated female offspring.The per cent of L-DOPA treated females displaying mounting behavior was also higher when compared with controls (100% vs 61%).Mount frequency of females displaying mounting behavior was also significantly higher in the L-DOPA offspring(60mg L-DOPA, 18 $\pm$ 2.6: saline, 9 $\pm$ 0.3).Parameters of masculine copulatory behavior in male offspring were altered inconsistently.

Lordotic behavior was not affected by L-DOPA treatment in female offspring, nor was a consistent effect in male offspring observed.

Thus manipulation of CNS neurotransmitter levels prenatally may have consequences evidenced in behavioral differences in adulthood.

168.9 MODEL PHENYLKETONURIA: BENEFITS OF A NEW THERAPEUTIC APPROACH. J. A. Beel*, L. J. Fisher*, L. S. Stodieck*, and M. W. Luttges, Department of Aerospace Engineering Sciences, University of Colorado, Boulder, CO 80309.

Recent studies (Luttges and Gerren, 1979) have shown that postnatal administration of α-methyl phenylalanine induces behavorial deficits in adult mice. Such deficits are characteristic of other animal models of phenylketonuria. Reductions in brain myelin proteins and changes in electrical recovery cycles were also observed. Based upon the hypothesis (Hughes and Johnson, 1978) that reduced brain protein synthesis produced by excess phenylalanine was the basis for phenylketonuria-like effects, studies of therapeutic reversal were attempted. Treatment with a mixture of large, neutral amino acids followed daily α-methyl phenylalanine treatments. Examinations of brain protein synthesis indicated restored levels of postnatal brain metabolism when the appropriate mixture of amino acids was administered. The restoration of synthesis levels was correlated with two additional observations: (1) electrophoretic and autoradiographic similarities in proteins isolated from various subcellular fractions and (2) variations in levels of ^{35}S-methionine uptake into intracellular pools. Following α-methyl phenylalanine treatment, those mice receiving a mixture of amino acids also exhibited normal daily increases in body weight. When tested behaviorally as adult mice, those mice which received amino acid therapy exhibited performance which differed significantly from the performance of mice which did not receive therapy. The treated mice also differed from control mice which received neither α-methyl phenylalanine nor therapy. These studies suggest that hyperphenylalaninaemia models of phenylketonuria depend upon imbalances in brain concentrations of large, neutral amino acids. Such imbalances are currently treated by lowering phenylalanine levels but may be approached by increasing the levels of other large, neutral amino acids. The latter approach, used here, appears to provide therapeutic benefits in the absence of dietary limitations common to the usual treatment of phenylketonuria.

168.10 BIOCHEMICAL CHANGES IN RATS WITH EXPERIMENTALLY INDUCED PHENYLKETONURIA (PKU). H. K. Berry, R. E. Butcher*, and P. G. Ruehlman*. Children's Hospital Research Foundation, Cincinnati, OH 45229.

Direct and indirect changes during early stages of brain development contribute to the pathogenic pattern in phenylketonuria (PKU).

We previously developed an animal model of PKU characterized by behavioral deficits persisting beyond the period of exposure to PKU-inducing diet. We used the model to examine biochemical changes at different stages of development. PKU was induced in pregnant rats by combined feeding of p-chlorophenylalanine, (PCPA), an inhibitor of phenylalanine hydroxylase, together with a slight excess of phenylalanine (PHE) in the diet. Fetuses were delivered by cesarian section at 22 days gestation. Brain weights of PKU fetuses were significantly lower than fetuses whose dams were pair-fed with PCPA or PHE alone. Maternal brain weights were unaffected.

Protein, DNA, cholesterol, cell number and cell size were lower in brains from fetal PKU animals than from any of the control fetuses. Cholesterol was the only maternal structural component which was lower in PKU dams than in control dams.

Reductions in brain serotonin were seen in both PKU and PCPA animals and, thus, were not attributable to the presence of excess PHE.

Brain PHE was elevated in PKU animals as expected; glycine was also elevated in PKU brains compared to controls. Valine, isoleucine, leucine, methionine and tryptophan were reduced in brains of PKU animals. These amino acids were fed to PKU rats to see if the deleterious effect of excess PHE could be overcome by supplying one or more essential amino acids. A supplement of valine, isoleucine, and leucine, fed to pregnant rats along with the PKU-inducing diet, resulted in fetal brain weights similar to those of pair-fed animals. Tryptophan and methionine feeding had no effect on fetal brain weights. PHE content of PKU fetal brains was reduced though there was no effect on plasma PHE levels. Supplementing the diet of PKU animals with valine, isoleucine, and leucine inhibited transport of PHE into brain and prevented the PKU-induced deficit in fetal brain weights. The data supported the hypothesis that brain protein synthesis was limited by lack of certain essential amino acids.

168.11 ION FLUCTUATIONS DURING NORMAL AND ABNORMAL BRAIN DEVELOPMENT - X-RAY MICROANALYSIS OF VENTRICULAR CONTENTS, TRANSPORTING EPITHELIA AND DEVELOPING NEURONS IN THE RAT. J.G. Chamberlain*+, R. Wróblewski* and Lars Edström* (SPON: M. Diamond). Cell Biology Section, Wenner Gren Institute and Karolinska Institute - Neurology, Stockholm, Sweden.++

Ultrastructural studies are not always reliable predictors of functional correlates. We report here important new data on electron probe x-ray microanalysis of developing brain cells and cerebrospinal fluid, demonstrating early regional and age differences with compartmentalization and electrolyte variability.

Embryonic and fetal brains from days 12-21 of pregnancy and one-day postnatal were quench frozen in liquid Freon 22 cooled with nitrogen (-180°), sectioned (14-16 μm) in the cold (-30°), and processed for x-ray analysis on carbon plates (JEOL 100C with Kevex). For comparison, brains of day 14 and 19 fetuses from mothers injected with niacin antagonist, 6-Aminonicotinamide (6-AN) (8 mg./Kg. b. wt. i. p.) on day 13 were similarly processed. Gelatin-embedded electrolyte standards allowed relative peak intensity (Rx) conversions to mM./Kg. dry wt. for cellular compartments. These and other data will be presented.

Relative concentrations of Na+ and Cl- differed in the cells of the cerebral cortex, ependyma, choroid plexus and cerebrospinal fluid (CSF) crystals. During cerebral development, Na+ and Cl- concentrations appeared to be correlated (ratio=1.0), while potassium was more related to phosphorus intensity (ratio=1.7). Sulphur was low in all compartments (R_S=1.0) and was considered as an internal control. K+ was inversely related to Na+/Cl- fluctuations within the choroid plexus epithelia. Sharp phase changes appeared in elemental composition at specific growth stages, e.g., days 14 and 19. The R_{Cl-} was highest (+23) in the ventricular crystals where its pattern followed most closesly that of K+. Na+ was also elevated in the ventricles early in development (i.e., day 12-13). When compared to controls, brain tissue and CSF crystals from 6-AN treated animals showed inverse changes in Na+, Cl- and K+ 2-24 hours after injection; five days later there still were differences (e.g., K+).

The relationship between the compartments of the early developing rat brain appear to continuously change, perhaps due to various growth processes reaching their respective peak and falling away at different times. The cerebral cortex, ependyma, choroid plexus and CSF appear to manifest individual ionic balances (e.g., Cl-) and marked cellular asynchrony early in embryogenesis (e.g., days 12-14). These balances are rapidly and markedly altered in prehydrocephalic young (6-AN treated).

+ Visiting professor - Univ. of the Pacific, San Francisco, CA

++ Support from Swedish Medical Council and NIH (GRS to U.O.P.)

168.12 AGE-DEPENDENT SUSCEPTIBILITY TO MICROWAVE HYPERTHERMIA INDUCED "FEBRILE" CONVULSIONS. D. L. Hjeresen, A. W. Guy* and J. Diaz. Dept. of Psychology, Univ. of Washington., Seattle, WA 98195.

Convulsions associated with fevers occur in an estimated 2 to 5% of children between 6 mo and 6 yrs of age. The neural mechanisms and clinical treatment of febrile convulsions are currently a matter of considerable debate. Previous animal models, based on raising ambient temperature or using electroconvulsive shock, have done little to resolve this issue. Rather, it seems likely that the stress induced by these methods, occuring at a critical period in brain development, could account for the deleterious effects on brain growth and behavior reported.

We have previously reported that when 13 and 17 day old rat pups are subjected to microwave (MW) hyperthermia convulsions can be induced. Further, we noted no deleterious effects of these convulsions on brain growth or behavioral performance. We also noted that pups tested at 17 days were less susceptible to convulsions than those tested at 13 days. This parallels the clinical observation that children beyond 6 yrs are no longer susceptible to febrile convulsions and suggests that a critical period exists in humans and rats for the induction of febrile convulsions. We test this hypothesis with the MW hyperthermia model developed in our laboratory.

Twelve litters of Long-Evans rats were culled to 8 pups each on Day 4 post-partum. One pup from each litter was assigned to each of the 8 exposure conditions: Exposed to MW energy (SAR=.378 W/kg per mW/cm^2 of input power) or sham exposed on Days 11, 13, 15, or 17. These days were selected since they represent the period of rapid brain growth in rats most comparable to the risk period for febrile convulsions in humans. Post-exposure behavior was scored on a 4 point scale with 4 representing severe clonic-tonic convulsions.

The results indicate a decreasing sensitivity to convulsions with increasing age. The mean (± SEM) behavioral ratings among pups exposed at 11, 13, 15, or 17 days were 3.17 ± 0.28, 2.54 ± 0.29, 1.67 ± 0.39 and 1.12 ± 0.13 respectively, indicating considerable convulsive behavior at 11 days, less so at 13 days and nearly normal behavior at 15 and 17 days, compared to controls. Activity measures, whole brain, cerebellar and brain stem weights and Day 21 body mass did not differ between groups either on the basis of exposure group or severity of convulsion. Analysis of additional behavioral parameters and neural biochemistry assays is in progress.

It is apparent from these initial results that humans and rats share a decreasing susceptibility to febrile convulsions during comparable periods of brain development. Further, it again demonstrates that MW hyperthermia promises to be a powerful research tool in the area of febrile convulsions.

169.1 SAFETY MARGIN OF NEUROMUSCULAR TRANSMISSION IN RAT EXTRAOCULAR MUSCLE. Yong I. Kim*, Daniel S. Zahm*, Humphrey H. Liu* and T. R. Johns. Dept. of Neurology and Div. of Biomedical Engineering, University of Virginia School of Medicine, Charlottesville, VA 22908.

Ocular symptoms such as ptosis and diplopia are present at onset in more than 65% of patients with myasthenia gravis (MG) and in approximately 10% of these patients the disease remains confined to extraocular muscles. Ocular myasthenia gravis may simply be a mild form of generalized MG, reflecting low titers of anti-acetylcholine receptor antibodies, or it could result from a different pathogenesis.

In an attempt to understand more about the disease process, we have examined the safety margin of neuromuscular transmission at singly innervated extraocular myoneural junctions (80-90% of the extraocular muscle fibers are singly innervated) and compared it with that in forelimb muscle of normal rats. In addition, rats with experimental autoimmune myasthenia gravis (EAMG) were also examined.

Using conventional intracellular recording and an on-line microcomputer system, experiments were performed on superior oblique extraocular muscle (EOM) and forelimb flexor digitorum longus (FDL) muscle in female Lewis rats.

In EOM of normal rats the amplitude of miniature end-plate potentials (MEPPs) was approximately twice the value recorded in FDL. This increase could be attributed to smaller EOM fiber diameter. MEPP frequency in the two muscles was not significantly different. MEPP and end-plate potential (EPP) waveforms were also similar in EOM and FDL. Impulse-evoked EPPs of EOM, measured in the presence of 0.6 µg/ml d-tubocurarine (dTC), were 36% smaller than the EPPs of FDL. Direct quantal content analysis, performed in the presence of 10 mM Mg^{++}, demonstrated that the mean number of ACh quanta released per nerve impulse is about 2 to 5 in EOM and about 10 in FDL.

The postjunctional sensitivity to dTC was measured as percent reduction in MEPP amplitude produced by low concentrations of dTC and was similar in the two muscles. When the MEPP amplitudes were measured in both EOM and FDL of the receptor-immunized EAMG rats, approximately the same percent reductions from control values were recorded in each muscle.

These results indicate that the rat extraocular muscle has a reduced safety margin of neuromuscular transmission which is likely due to a decrease in the number of ACh quanta released from motor nerve terminals and probably involves no postsynaptic factor. We suggest that this reduced safety margin should be demonstrable in human EOM and it is partially responsible for the early appearance of ocular symptoms in MG patients. (Supported in part by a center grant from the Muscular Dystrophy Association of America)

169.2 SINGLE FIBER ELECTROMYOGRAPHIC STUDIES IN MULTIPLE SCLEROSIS, J.K.Baruah* and B.O. Khatri*, (Spon: M.P. McQuillen) Department of Neurology, The Medical College of Wisconsin, 9200 W. Wisconsin Avenue, Milwaukee, WI 53226.

Forty percent (McAlpine et al, Multiple Sclerosis, 1st ed., Livingston 1955) of patients with multiple sclerosis (MS) complain of weakness in extremeties, often present as tiredness and fatiguibility. The later significantly get worse on physical exertion. In large majority of these patients, the weakness is secondary to demyelination of corticospinal tract. However, in some the clinical dysfunction of corticospinal tract is lacking, but they still may complain of tiredness or fatigue. The possibility that this complaint might be secondary to defective neuromuscular transmission was addressed with single fiber electromyography (SFEMG) in addition to routine EMG in a group of patients with chronic progressive MS. Twenty-one patients (M = 10, F - 11) were selected for this study and all of them had significant degree of weakness in the extremeties. The routine peripheral nerve conduction, needle EMG and repeatitive stimulation (ulnar nerve at 3HZ) studies were normal in all patients. The SFEMG studies of extensive digitorum brevis muscle at multiple sites revealed prolongation of jitter values in 15/21 patients. The range of mean consecutive jitter difference in these patients was 56 - 285 µsec (normal 22 - 52 µsec). Blocking of second potentials was detected in 8/15 patients with abnormal jitter values. Serum anti-acetylcholine receptor antibody was not detected in any patient. These findings suggest that in many patients with MS, who complains of fatigue, neuromuscular transmission may be defective. The mechanism of this effect is not known. Studies are underway to ascertain whether the defect is repaired as the symtom improves.

169.3 QUANTUM CONTENT OF END-PLATE POTENTIALS IN EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS. Humphrey H. Liu*, Yong I. Kim* and T.R. Johns (SPON: G.R. Hanna). Dept. of Neurology and Div. of Biomedical Engineering, University of Virginia School of Medicine, Charlottesville, VA 22908.

Myasthenia gravis (MG) is a disease of the neuromuscular junction caused by an immune response which results in the production of autoantibodies against acetylcholine receptors (AChRs). The disease is characterized by a reduction in the number of functional AChRs, leading to a postsynaptic impairment of neuromuscular transmission. A recent study of intercostal muscle biopsies from patients with MG (Cull-Candy et al., J. Physiol. 299:621-638,1980) indicates, however, that presynaptic changes also occur at the myasthenic neuromuscular junction. The mean number of transmitter quanta (m) released from MG motor nerve terminals is significantly greater than normal, especially when $[Ca^{++}]_o$ is low. We studied the transmitter release process in rats with experimental autoimmune myasthenia gravis (EAMG) in order to determine whether a similar increase in m is demonstrable.

EAMG was induced in female Lewis rats by immunization with purified Torpedo AChR protein suspended in Freund's complete adjuvant. Age-matched control rats received saline and adjuvant. Intracellular recordings were made in vitro on flexor digitorum longus muscles 45 to 189 days after the initial immunization. In the presence of 3 mM $[Mg^{++}]_o$, mean quantal content was estimated at three different values of $[Ca^{++}]_o$, 0.25, 0.4 and 0.6 mM. At each end-plate both direct and indirect variance methods were used to calculate m and 10 to 15 end-plates were sampled in each muscle. All biopotentials and quantal release parameters were analyzed on-line using a microcomputer system.

In the range of $[Ca^{++}]_o$ studied, the mean values of m in EAMG were found to be 25 to 50% greater than normal. We found a linear relationship between log m and log $[Ca^{++}]_o$ which showed a slope of 3.2 in EAMG and 3.3 in normal muscle. The increase in evoked transmitter release, however, was not found in all receptor-immunized animals studied; a wide variation appears between different animals. The increased quantal transmitter release was also found to vary as a function of disease duration. Animals sacrificed after a prolonged period since initial immunization seemed to show a larger increase in m.

These results demonstrate a presynaptic change in the transmitter release process associated with the development of EAMG in rats. Although the mean quantal content in EAMG does not seem to increase to the extent described in human MG, such a presynaptic change is an important factor to consider in determining the overall severity of neuromuscular blockade. (Supported in part by a center grant from the Muscular Dystrophy Association of America)

169.4 IMPAIRED NEUROMUSCULAR TRANSMISSION IN STREPTOZOTOCIN INDUCED DIABETES. G.A. Ruskan* and A.B. Drakontides Dept. of Anatomy, New York Medical College, Valhalla, NY 10595.

The manifestations and consequences of diabetic peripheral neuropathies have been well documented. Although morphological studies have implicated the motor nerve terminal as a prime site of lesion, few studies have examined neuromuscular transmission in the diabetic state.

The in vitro phrenic nerve hemidiaphragm preparation from 3 and 6 month streptozotocin (STZ) diabetic rats (50 mg/kg IV) rats, and age matched controls, were used to evaluate neuromuscular function. Parameters studied were: 1) indirect isometric twitch tension 2) post-tetanic potentiation (PTP) following nerve stimulation at frequencies of 10-100 HZ 3) fatique time following continuous stimulation of nerve at 10 HZ 4) potentiating effects of edrophonium. The data were compiled from 300-400 gm diabetic rats of 3 and 6 month duration with mean blood glucose values of 401 ± 10 gm/dl and compared to age matched controls of the same weights (blood glucose 105 ± 2 mg/dl).

Indirect twitch tension in both diabetic groups was not significantly different from controls. PTP could be elicited at all frequencies tested; however, the magnitude of potentiation was reduced, the greater reduction being evident in 6 month diabetic preparations (3 month 28%, 6 month 53% decreased PTP). This decrease is not attributable to a dysfunction of muscle contractile mechanisms, since direct stimulation of diabetic diaphragms elicited the same magnitude of response as seen in control preparations. PTP in rat diaphragm primarily reflects a neurogenic post-tetanic repetition of slow muscle nerve fibers, and this decrease in PTP suggests a loss of functional capabilities of these nerve fibers. Both diabetic groups fatiqued more rapidly than controls (control 151 ± 7 sec, 3 month 87 ± 6 sec., 6 month 104 ± 9 sec.). While this decrease might reflect a metabolic deficiency, an alternative explanation might be impairment of neural transmission. Degenerating motor nerve terminals have been demonstrated in human diabetes and animal models which would support this contention. In addition a reduction in cholinesterase activity is evident. In the presence of 10^{-5} and 10^{-6} g/ml concentrations of edrophonium, single indirect tiwtch responses were significantly enhanced in 3 month diabetics (32%, 45% respectively). This effect probably reflects the diminished cholinesterase activity which increased the concentration of ACh available at the neuromuscular junction.

These studies suggest that the neuromuscular junction is an initial site affected in STZ induced diabetes. Moreover, deficits of neuromuscular transmission are evident under conditions equivalent to a moderate degree of sustained hyperglycemia.

169.PO THE USE OF COMPREHENSIVE NERVE CONDUCTION STUDIES TO DELINEATE NERVE ROOT COMPROMISE NON-INVASIVELY AND ITS ADVANTAGES OVER CONVENTIONAL NEEDLE ELECTROMYOGRAPHY. R.A. Cyrulnik*(SPON:D. Becker)
Lab for Diagnostic Non-Invasive Analysis, Huntington Beach, CA92647

Using bipolar surface stimulation and recording techniques, late F-wave and H-wave responses were measured together with orthodromic sensory nerve conduction latencies and amplitudes in the distribution of cervical and lumbosacral nerve root innervations of patients presenting with clinical signs of radiculopathy. Distal peripheral nerve conduction velocities were determined in each case to rule out peripheral neuropathy as a contributing factor to clinical or electrodiagnostic findings. Absolute latency values (upper limit 32.0 msec. in cervical region, 54.5 msec. in lumbosacral region) were used as criteria for normality as well as relative comparison with conduction in adjacent roots (upper limit 2.0 msec.) for F-wave determinations, as well as for H-wave evaluation (upper limit of normal 32.0 msec., bilateral difference under 2.0 msec.). Standard absolute criteria were used for the various sensory nerve conduction values. The results showed excellent correlation with the clinical findings as well as conventional needle electromyography with the following advantages: (1)Significantly greater patient acceptance due to marked reduction of the pain of the procedure; (2)Significantly earlier detection of pathology because axonal degeneration is not a prerequisite for abnormal conduction; (3)Enhanced sensitivity since myelin sheath compression alone will alter conduction; (4)Availability of data regarding sensory abnormalities not detectable by conventional intramuscular EMG; (5)Detection of double crush syndromes since distal conduction data must be measured; (6)Improved correlation with the clinical course of the patient associated with rapid response of conduction changes to resolution of nerve root compression; (7)Direct determination of conduction in the roots themselves rather than by inference from spontaneous muscular activity. This technique has proven a valuable adjunct to needle electromyography both as a screening procedure as well as in those cases where conventional EMG alone cannot provide sufficient data to delineate all of the clinical findings. It can also be used in those situations where the patient cannot tolerate the pain of needle electromyography or are in such muscular spasm and/or unable to relax as to render the detection of spontaneous muscular activity impossible on needle EMG, as well as where the risk of infection from an invasive procedure contraindicates needle EMG. The above advantages make this technique an attractive alternative to needle electromyography in early screening of radiculopathy as a non-invasive form of EMG. Examples will be given.

170 SYMPOSIUM

ALZHEIMER'S DISEASE AND THE CHOLINERGIC INNERVATION OF NEOCORTEX BY THE NUCLEUS BASALIS. M-M. Mesulam, Beth Israel Hospital and Harvard University (Chairman); J. Axelrod, NIH-NIMH; K. Krnjevic, McGill University; M.J. Kuhar, John Hopkins University; P. Davies*, Albert Einstein Medical College, Bronx, N.Y.; J.T. Coyle, Johns Hopkins University; D.L. Price*, Johns Hopkins University.

Mammalian neocortex contains presynaptic cholinergic elements as well as neurons that respond to cholinergic agonists. A major portion of the cholinergic innervation in neocortex arises from neurons located in the nucleus basalis of Meynert. This cholinergic corticopetal pathway appears to be selectively involved in Alzheimer's Disease.

In this symposium, J. Axelrod will provide introductory remarks. K. Krnjevic will discuss the physiology of neocortical cholinergic transmission. M.J. Kuhar will describe the distribution and pharmacological identity of cholinergic receptors in neocortex. P. Davies will provide evidence for a selective loss of neocortical presynaptic cholinergic markers in Alzheimer's Disease. M-M. Mesulam will provide histochemical evidence to show that the nucleus basalis of Meynert is a major source for the cholinergic innervation of neocortex. It will also be shown that different parts of the nucleus basalis project to different portions of the cortical mantle. J.T. Coyle will show that there is pharmacological evidence for corticopetal cholinergic innervation originating in the nucleus basalis. D.L. Price will show that the nucleus basalis is preferentially involved in Alzheimer's Disease.

The identification of this cholinergic pathway as a site of special predilection provides a basis for further research on the phenomenology, etiology and treatment of Alzheimer's Disease.

171 SYMPOSIUM. SINGLE CHANNEL RECORDING. Charles F. Stevens, Yale (Chairman); Vincent E. Dionne*, UCSD; Steven Siegelbaum*, Columbia; Gary Yellen*, Yale; Harunori Ohmori*, UCLA; David Tank*, Cornell.

During the past year, the Neher patch recording method has been applied to a variety of new systems, and has provided insights into neuronal function at the level of single channels. Talks at this symposium will focus on some of the recent advances made with this method, and highlight some of its advantages.

The acetylcholine receptor remains the best studied channel protein, but the most widely used approach, fluctuation analysis, has not provided information about states preceding the open one. Statistical analysis of single channel currents yields inferences about pre-opening steps (Dionne).

5-HT produces, through a cAMP dependent protein phosphorylation step, a decrease in molluscan potassium currents. The mechanism of this modulatory effect has been investigated at the level of individual potassium channels (Siegelbaum).

Potassium currents play a central role in regulating neuronal electrical activity. Single channel analysis reveals unexpected complexities in the behavior of channels responsible for these currents (Yellen).

GH cells, from a pituitary derived cell line, depend on calcium currents for the regulation of secretion. The function of the channels linking excitation to secretion has been analyzed for these cells (Ohmori).

Planar bilayers have typically been used in attempts to reconstitute channels for elecrophysiological study. Torpedo chloride channels have been reconstituted in lipisomes and studied by the Neher method, thereby offering an advantageous alternative to the usual planar bilayer approach (Tank).

172 SYMPOSIUM. SEX HORMONES AND NEURAL DEVELOPMENT: IMPLICATIONS FOR THE GENESIS OF SEXUAL DIFFERENTIATION. C.D. Toran-Allerand, Columbia Univ. Coll. of Physicians and Surgeons (Chairman); W.T. Greenough, Univ. of Illinois; D.B. Kelley, Columbia Univ.; A.P. Arnold, Univ. of California, Los Angeles.

In many vertebrates sex differences in various neural structures and of numerous neuroendocrine and behavioral responses results from exposure of the CNS to endogenous sex steroids during restricted developmental periods. The cellular mechanisms involved appear to represent a complex interaction of hormonal and non-hormonal factors, the relative contributions of each varying among species. This symposium will consider the broad range of structural dimorphisms which are found in a wide variety of species and the in vivo and in vitro approaches which have been used to try to characterize and analyze the hormonal and non-hormonal contributions to the ontogeny of sexual differentiation. Greenough will review and compare the various morphological differences which have been described in the brain of various mammals; their relationship to the postulated existence of sexually dimorphic interneuronal connectivity patterns and the possible mechanisms involved in their origin. Kelley will consider the hormonal and non-hormonal factors in the differentiation of the sexually dimorphic laryngeal motoneurons of the frog, Xenopus laevis, focusing on the apparent differences in the developmental mechanisms controlling neuronal number (non-hormonal) and those controlling neuronal size (hormonal). Arnold will discuss the androgen-dependent sexually dimorphic neuromuscular systems of the bird and rat, emphasizing the different types of developmental processes influenced by androgen; the existence of distinct critical periods for each effect and the possible mechanisms involved. Toran-Allerand will consider the cellular expression of the steroidal effects in cultures of the developing CNS as one approach to elucidating the morphogenetic basis for the observed sexual dimorphism of the adult.

173 WORKSHOP

SPRINGING INTO ACTION: MECHANISM AND FUNCTION OF SPRING-LIKE PROPERTIES OF NEUROMUSCULAR SYSTEMS. J.C. Houk (Chairman, Northwestern Univ. Med. Sch.), J.A. Hoffer (NIH), W.Z. Rymer (Northwestern Univ. Med. Sch.), N. Hogan (MIT), R.B. Stein (Univ. of Alberta), T.A. McMahon (Harvard Univ.).

An analogy between the musculature and mechanical springs is a common theme of several recent developments in the field of motor control. This workshop was organized to evaluate origins of spring-like properties and to discuss their functional significance in normal motor control. Houk will introduce the topic by contrasting the intrinsic mechanical stiffness of skeletal muscle with the stiffness present when spinal reflexes are operative. The former derives largely from the elasticity of cross bridges whereas the latter is an emergent property dependent on neural feedback. Hoffer will review data showing that reflexes compensate for variations in intrinsic stiffness which result from stretch or from alterations in initial conditions. Variations in reflex gain are apparent from these data, but they may represent fixed nonlinearities rather than actions of a stiffness control system. Hoffer supports the notion of stiffness regulation, though not that of stiffness control. Rymer will explore potential mechanisms for stiffness regulation by comparing motor unit and muscle receptor activity with observed indices of reflex action. He will suggest that recruitment contributes more compensation than does rate modulation and that nonlinear feedback from primary spindle receptors is the major source of compensation for stretch-induced failures in stiffness. Data will be presented showing that force feedback contributions to stiffness regulation are not prominent in reduced animal preparations. Hogan will discuss the role of viscoelasticity (dynamic stiffness) in controlling voluntary limb trajectories. Data obtained for trajectory tracking by monkey subjects will be compared with optimal models, and questions of control strategy and central programs versus peripheral feedback will be addressed. Stein will bring us back to the issue of reflex gain modulation and its potential for stiffness control. In support of the latter concept he will describe stiffness measurements obtained from locomoting mesencephalic cats and results obtained in human subjects during tasks contrasting rigid positional control with compliant force control. McMahon will relate the concepts and results presented by previous speakers to walking and running performance in man. Spring models will be used to analyze running on a circular path, in enhanced gravity and on a compliant surface. The role of elastic storage in locomotion will also be evaluated.

174.1 BEHAVIORAL AND PHYSIOLOGICAL CHARACTERIZATION OF NON-NARCOTIC ANALGESIA PRODUCED BY CHOLINERGIC MICROINJECTION IN THE PARA-BRACHIAL REGION OF THE CAT. Y. Katayama*, S. M. Carlton*, D. P. Becker and R. L. Hayes (SPON: W. I. Rosenblum). Div. of Neurosurgery, Virginia Commonwealth Univ. Richmond, VA 23298.

Two guide tubes (26 gauge) were chronically implanted bilaterally through the cerebellum of 20 cats. After recovery from surgery, awake, unanesthetized cats were loosely restrained in canvas bags. Carbachol (0.3-0.6 µg in 0.15-0.3 µl of 0.9% saline, bilaterally, pH 7.4) was microinjected at 4 day intervals through 33 gauge cannulae into various brain stem loci. Four behavioral tests qualified nociceptive responses: tail withdrawal from noxious heat, applications of calibrated pinch to the extremities, subcutaneous injections of formalin and brief applications of alligator clips to the extremities. Additional tests examined other sensory and motor abilities, including studies of righting and placing reflexes, startle responses and locomotion. Over 50 sites were studied. Analgesic sites (n=20) were distributed around the dorsolateral half of the brachium conjunctivum (BC) from the marginal nucleus of BC, caudally, to the parabrachial reticular formation, rostrally. Some medial sites in this region produced analgesia associated with reduced spontaneous activity. The majority of analgesic animals showed marked suppression of all nociceptive behaviors in the absence of any evidence of motor or other sensory disturbances. Microinjection of 0.6 µg carbachol bilaterally produced suppression of the tail-flick reflex comparable to that produced by morphine (2.0 mg/kg, s.c.). Atropine either microinjected (1.0 µg in 0.5 µl, bilat., n=4) or systemically administered (0.5 mg/kg, i.v., n=8) after microinjection of carbachol significantly antagonized carbachol analgesia. Mecamylamine had no effect on carbachol analgesia either when microinjected (0.3 µg in 0.2 µl, bilat., n=4) or systemically administered (0.5 mg/kg, i.v., n=4) after carbachol microinjection. Naloxone (2.0 mg/kg) administered either before carbachol microinjection (i.p., n=4) or after carbachol analgesia (i.v., n=4) had no effect. No analgesia was produced by morphine microinjection (25 µg in 0.5 µl, bilat., n=4) into the same sites associated with carbachol analgesia.

These data suggest that: (1) supraspinal sites in the cat parabrachial region can selectively modulate the integration of nociceptive responses at the level of the spinal cord, (2) activity at these sites is influenced by muscarinic cholinergic rather than opiate mechanisms. Supported by NIH Grant #NS12587.

174.2 EVIDENCE FOR THE INVOLVEMENT OF HISTAMINE IN STRESS ANALGESIA. G.W. Terman*, J.W. Lewis, and J.C. Liebeskind, Department of Psychology, University of California, Los Angeles, CA. 90024.

In the past decade, much evidence has accumulated in support of the existence in the brain of a system whose natural function is to decrease the perception of pain. Numerous observations that stress can reduce pain responsiveness have suggested that stress may be a natural trigger for activating this system. Stress-induced analgesia, however, does not appear to be a unitary phenomenon. We have found that by varying the temporal parameters of footshock stress, opioid mediated and nonopioid mediated analgesia can be differentially produced (Lewis et al., 1980). Although several chemical mediators for the opioid form of stress analgesia have been suggested, the neurochemistry of the nonopioid form is less well understood. In this regard, we have recently reported that nonopioid stress analgesia, while resistant to serotonin, norepinephrine, and dopamine receptor antagonists and depletors, is attenuated by the histamine H_1 receptor antagonist, diphenhydramine (Terman et al., 1982). The present study was undertaken in an attempt to extend these results suggesting a role for histamine in stress analgesia.

Twenty male Sprague-Dawley rats were randomly divided into two groups. One group was injected with saline (i.p.), the other with α-fluoromethylhistidine (αFMH) (100 mg/kg, i.p.). αFMH is an irreversible inhibitor of L-histidine decarboxylase and has been seen to disrupt histamine synthesis in the rat liver (Kollonitsch et al., 1978) and the mouse brain (Garbarg et al., 1980). In mice, this disruption is particularly effective in depleting the rapidly turning over neuronal stores of histamine. Eight hours after drug or saline injection, each animal was tested for baseline pain responsiveness using the tail-flick test. Rats were then subjected to footshock stress according to the parameters known to produce nonopioid analgesia (3 min, 2.5 mA, 60 Hz sine waves on continuously). Recording of tail-flick latencies began one min after stress and continued at one min intervals for 12 min. Animals given αFMH, while not differing significantly from controls on baseline tail-flick latencies, were significantly less analgesic than controls after footshock ($p < .05$). These results support our previous findings suggesting that histamine plays a significant role in mediating the nonopioid form of stress-induced analgesia. (We thank Merck, Sharp, and Dohme for the generous gift of α-fluoromethylhistidine. Supported by NIH grant NS07628. G.W.T. was supported by training grant MH15795.)

174.3 CROSS-TOLERANCE BETWEEN OPIOID MEDIATED STIMULATION-PRODUCED AND STRESS-INDUCED ANALGESIA. E.R. Penner*, G.W. Terman*, and J.C. Liebeskind. Dept. of Psychology, University of California, Los Angeles, CA. 90024. (SPON: D.B. Lindsley)

Cross-tolerance experiments have proven to be a useful tool for demonstrating separate activation of a common neurochemical pathway. For example, analgesia produced by electrical stimulation of the midbrain periaqueductal gray matter has been found to manifest tolerance and cross-tolerance with morphine analgesia (Mayer and Hayes, 1975). This finding suggested that stimulation-produced analgesia (SPA) involves activation of the same endogenous pain-inhibitory system as that activated by morphine. More recently, stress has been suggested as a natural activator of this system. Numerous stressors have been reported to produce analgesia. We have demonstrated that naloxone-sensitive (opioid) and -insensitive (nonopioid) stress analgesia can be differentially produced by varying the temporal parameters of the footshock stress administered (Lewis et al., 1980). Moreover, the opioid but not the nonopioid form of stress analgesia shows tolerance and cross-tolerance with morphine (Lewis et al., 1981). Like stress analgesia, SPA has also been found to be mediated by both opioid and nonopioid mechanisms depending on the specific location of the stimulation site (Cannon et al., in press). Stimulation sites in ventral PAG cause naloxone-sensitive SPA, whereas sites in dorsal PAG cause naloxone-insensitive SPA. In this study we investigated whether the opioid forms of SPA and stress analgesia are mediated via the same pain inhibitory system, by testing for cross-tolerance between them.

Twenty-four male Sprague-Dawley rats were implanted with quadrupolar stimulating electrodes, with bipolar electrode sites in dorsal and ventral PAG. One week after surgery animals were tested for dorsal and ventral baseline SPA current thresholds, then divided randomly into two groups. For the following 14 days one group received footshock daily using parameters known to produce opioid analgesia (2.5 mA, 1 sec pulse/5 sec; 60 Hz sine waves for 20 min); the other group served as non-shocked controls. On the 15th day animals were again tested for dorsal and ventral SPA thresholds. In non-shocked animals baseline threshold values were not found to differ from final threshold values at either dorsal or ventral electrode sites. Shocked animals, however, while not showing any differences between baseline and final SPA thresholds for dorsal placements, did have final ventral thresholds that were significantly increased above baseline ($p < .01$). The present findings support the view that the opioid forms of SPA and stress analgesia are mediated by a common opioid pathway. (Supported by NIH grant NS07628. G.W.T. was supported by training grant MH15795.)

174.4 THE EFFECT ON NOCICEPTION IN RATS OF DIRECT APPLICATION OF SUBSTANCE P AND 5-HYDROXYTRYPTAMINE INTO THE NUCLEUS RAPHE MAGNUS. DONALD E. WILLIAMS. DEPARTMENT OF PSYCHOLOGY, UNIVERSITY OF GEORGIA, ATHENS, GEORGIA 30609.

The nucleus raphe magnus (NRM) contains serotonergic neurons which project to the spinal cord dorsal horn via the dorsolateral funiculus where they inhibit pain-conducting neurons. The NRM receives a projection from the periaqueductal gray (PAG), and NRM neurons are activated by electrical stimulation or morphine microinjections in the PAG.

Although PAG stimulation elicits responses from NRM neurons, little is known about the neurotransmitters that mediate these responses. Serotonergic projections from various brainstem nuclei to NRM have been described, however the effects of serotonin (5-HT) on NRM neurons is unknown. Substance P (SP) has been found in PAG cell bodies and in axon terminals within NRM. SP and 5-HT coexist in NRM neurons and autoreceptive, reuptake, and storage mechanisms for SP and 5-HT have been suggested. Single cell recordings have shown SP to excite NRM neurons. Thus, SP and 5-HT have been implicated as two possible modulatory transmitters in NRM-mediated analgesia. This hypothesis was tested using a tail flick response to heat following injection of SP or 5-HT into the rat NRM.

Since all NRM sites have been shown upon stimulation to suppress tail flick responses equally well, all injections were made through cannulae guides implanted in the dorsal NRM. Each injection was performed manually over 30sec. using a microsyringe and injector fitted to the cannula guide. 27 male rats (9/group) were tested for tail flick latency before (baseline), and 25 (T25) and 60 (T60) minutes after NRM injections of SP (2.5µg/0.5µl), 5-HT (10.0µg/0.5µl), or vehicle (saline; 0.5µl). 9 non-operated control subjects were tested similarly.

At baseline, there were no differences between groups. At T25, 5-HT subjects displayed longer latency scores ($p<0.01$). SP T25 and 5-HT T60 subjects approached showing significantly longer latencies ($p<0.10$). 3 additional subjects received a higher SP dose (5.0µg/0.5µl) and were tested without significant results.

The results suggest that SP at the given doses does not produce significant analgesia when applied directly to NRM, questioning its role as an important transmitter in NRM-mediated analgesia Rather, SP may act as a neuromodulator within NRM. The data also suggest that 5-HT may be important in endogenous analgesia by exerting an excitatory effect on NRM neurons; an effect similar to its hypothesized action on enkephalinergic interneurons in the spinal cord dorsal horn. The data in general are in accord with previous conclusions that serotonergic afferent NRM projections may subserve the descending pain control system and thus deserve further study.

174.5 MONOAMINES AS MEDIATORS OF THE ANALGESIC ACTION OF BACLOFEN. J.Sawynok, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

Baclofen, an antispastic agent, can produce analgesia by actions at both spinal and supraspinal sites. Baclofen is similar to morphine in that it is effective administered into the periaqueductal grey while analgesia resulting from systemic injection is reduced by spinal cord section. Descending monoaminergic pathways are implicated in the analgesic action of morphine. In the present study, the role of monoamines in the analgesic action of baclofen was investigated.

Analgesia was evaluataed using the tail flick and hot plate tests and male Sprague Dawley rats (150-250g). Both reserpine (5 mg/kg, 18 hrs pretreatment) and α-methyl-p-tyrosine (250 mg/kg, 18 hrs pretreatment), which deplete brain catecholamine content, markedly potentiated the analgesic action of baclofen (10 mg/kg) administered intraperitoneally at all time intervals during the 3 hr observation period. The receptor antagonists phentolamine (5 mg/kg) and haloperidol (0.5 mg/kg) produced a similar potentiation. Reserpine, phentolamine and haloperidol alone were without significant effect on reaction latency, while α-methyl-p-tyrosine produced a slight but significant increase in tail flick latency. Agents interfering with serotonergic function by depletion (p-chlorophenylalanine, 300 mg/kg, 48 hrs pretreatment) or receptor antagonism (methysergide, 5-10 mg/kg) did not consistently alter the analgesic action of baclofen. When baclofen was administerd intrathecally (0.5 µg), reserpine and α-methyl-p-tyrosine did not potentiate analgesia for one hour following injection, but did to a mild degree at later time intervals. In these experiments, it is likely that baclofen initially was localized to the spinal cord but subsequently spread to supraspinal sites.

These results indicate that a decrease in catecholaminergic function enhances the analgesic action of baclofen, probably by an action at supraspinal sites. Serotonin does not appear to be critical for the expression of analgesia.

174.6 LATERAL HYPOTHALAMIC REWARD SITES YIELDING POSITIVE AND NEGATIVE STIMULATION-BOUND FEEDING RESPECTIVELY ATTENUATE AND POTENTIATE AVERSION. P. E. Simson* and E. E. Coons* (SPON: L. Brown). Dept. of Psychology, New York University, New York 10003.

In rats electrical stimulation of the lateral hypothalamus (LH) at reward sites which also elicited stimulation-bound feeding decreased lever pressing for 3-second interruptions of otherwise continuous trains of pulses to the pain-implicated nucleus reticularis gigantocellularis (NGC). This ameliorative effect occurred at currents both above and below those necessary to support classical self-stimulation and increased in magnitude with increases in current over a considerable microamperage range. By contrast, electrical stimulation at LH reward sites which did not elicit stimulation-bound feeding increased lever pressing to escape NGC stimulation. This potentiation of escape also occurred at currents both above and below those necessary to support self-stimulation and increased with increases in LH current amplitude. Thus, currents supporting self-stimulation behavior can have opposite effects on NGC-escape depending on whether or not there is also elicitable from the LH site an appetitive motivational state. These findings amplify previous reports from our laboratory (Carr, K. D. and Coons, E. E., Science 215, 1516, 1982) that the LH aversion-ameliorative mechanism is directly dependent on motivational rather than reward mechanisms activated by the LH stimulation. The existence of aversion-ameliorative mechanisms associated only with reward sites yielding feeding has implications for the observation that an escape component in self-stimulation behavior (reward-escape) is not associated with such sites as it is with others.

174.7 MEDIAL FOREBRAIN BUNDLE LESIONS IN THE RAT: THE EFFECTS OF STRAIN AND SHOCK PARAMETERS ON FOOTSHOCK SENSITIVITY. C. E. Lints and N. Mogharreban*. Dept. of Psychology, Northern Illinois Univ., DeKalb, IL 60115.

Bilateral medial forebrain bundle (MFB) lesions have been reported to produce an increased sensitivity to footshock in rats that has been interpreted as hyperalgesia and been related to the forebrain serotonin (5-HT) depletion produced by these same lesions. After difficulty replicating the effect in this laboratory, it was shown to be sensitive to the shock parameters used in testing (Lints, Nenja and Miller, Neurosci. Abstr., 5: 613, 1979). To examine the possibility that the strain of rat is also important, this experiment was designed to compare the effects of various shock parameters on footshock sensitivity in two different strains of rats with MFB lesions.

Sham-operated and MFB-lesioned rats of both the Holtzman Sprague-Dawley (Albino-MFB) and Long Evans (Hooded-MFB) strains were retested for footshock sensitivity using the flinch-jump technique, a custom-made shocker, and 3 different shock durations (.05, .1 and .2 sec). The animals were then tested with a Coulbourn (Model E13-33) shocker using the .1 sec shock duration. Both shockers were constant current devices that delivered ac current to the grids of the test chambers, and the rats were always retested in the same chambers. Following behavioral testing the forebrains were assayed for 5-HT and norepinephrine (NE) levels and the brainstems were used for histological verification of the locus and extent of the lesions.

The lesions were well-localized, and produced comparable forebrain 5-HT and NE depletions. With the custom shocker the Albino-MFB rats showed significantly lower flinch and jump thresholds than their controls with all 3 shock durations. The Hooded-MFB rats had lower flinch thresholds than their controls only with the .05 sec duration shocks, and lower jump thresholds only with the .1 sec duration shocks. With the .1 sec shocks the Hooded-Controls had significantly lower jump thresholds than the Albino-Controls. When retested with the Coulbourn shocker, the Albino-MFB rats were not different from their controls with respect to either flinch or jump thresholds and the Hooded-MFB rats showed significantly elevated flinch and jump thresholds. The shocker effect was replicated for both strains of rats in a separate experiment. The results of these experiments are discussed in terms of the differential sensitivity of the two strains of rats to footshock and the relationship of this measure of footshock sensitivity to pain sensitivity.

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174.8 ENDOGENOUS ANTINOCICEPTION IN THE PREGNANT RAT. June L. Dahl†, Stephen T. Tiffany* and Timothy B. Baker*. Departments of Pharmacology† and Psychology, University of Wisconsin - Madison, Madison, WI 53706.

Although there is considerable evidence for endogenous mechanisms of pain inhibition, the physiological and psychological factors which control their activation have not been clearly elucidated. It has been shown that a variety of stressors produce analgesia in experimental animals. Gintzler has reported (Science 210, 193, 1980) that response to painful stimuli is reduced during pregnancy and parturition in the rat. We have initiated a series of experiments designed to define the neurochemical basis of the altered pain response during pregnancy. Sprague Dawley rats, with ad lib food and water, were individually housed and maintained on a 12-hour, light-dark cycle. In two initial studies, pregnant and nonpregnant rats did not differ in antinociception assessed by the tail flick procedure. This finding was substantiated in a third study in which measurements were made from the 5th day of gestation until one day after parturition. Data were analyzed by analysis of variance with the Greenhouse-Geisser correction for repeated measures. Across test days, a repeated measures analysis of variance revealed no significant differences in tail flick latencies between the pregnant and nonpregnant groups. Neither were there any significant interactions between pregnancy and other parameters, e.g., test day, time of test, p's > 0.05. However, when antinociception was assessed by an automated flinch/jump procedure, there was an overall group difference between pregnant and nonpregnant animals, $F(1,17) = 13.35$, $p < 0.01$. There was also a significant interaction between pregnancy and shock level such that group differences were greater at the higher shock levels $F(4,68) = 4.69$, $p < 0.01$, a finding typical of opiate induced analgesias. At one day postpartum, groups did not differ in flinch/jump response. Our flinch/jump data, and the absence of differences with the tail flick measure, indicate that the ability to demonstrate antinociception accompanying pregnancy is dependent on the nature of the analgesia assessment procedure. We are currently conducting studies designed to reveal whether pregnancy-induced antinociception is naloxone reversible and whether it is associated with changes in levels of brain opiate peptides as determined by radioimmunoassay. Supported in part by funds provided by the Research Committee of the Medical School (JLD) and by NIDA grant RO1DA02729-02 (TBB).

174.9 POTENTIATION OF COLD SWIM STRESS ANALGESIA BY DIAZEPAM. Donald S. Leitner and Dennis D. Kelly Dept. of Psychiatry, Columbia Univ. and NYS Psychiatric Institute, New York, New York.

Acute exposure to a stressor can elevate pain thresholds for a period that outlasts the exposure. Stress appears to be a necessary, if not sufficient, condition for inducing analgesia. Thus, physiological manipulations known to alter the experience of stress should modulate the analgesia prompted by an effective analgesic stressor. Diazepam, a minor tranquilizer, releases behavior suppressed by punishment and attentuates a number of stress-related, aversively controlled behaviors, such as pain-induced fighting and avoidance. We examined whether it might similarly attenuate the prolonged analgesia induced by a forced swim in cold water. In fact, it had the opposite effect.

In Exp I 11 rats were exposed to six conditions. In the first two, the rats were injected IP with 2mg/kg diazepam (DZP) either (1) 30 mins before, or (2) immediately following, a 3.5-min swim in 2°C water; flinch-jump testing occurred 30 mins following the swim. In two no-stress conditions the rats received similar DZP injections either (3) 30 mins or (4) 60 mins before testing. In two drug-free baseline conditions the subjects were exposed (5) to a cold-water swim 30 mins before testing or (6) to flinch-jump testing alone. Each rat was exposed to all six conditions in a Latin square sequence. DZP administered either before or after a swim significantly increased the amount of stress analgesia (conditions 1 and 2 vs 5). Yet, DZP by itself had no affect upon thresholds at the same post-injection intervals (3 and 4 vs 6). Thus DZP potentiates stress-induced analgesia without affecting normal pain thresholds and does not have to be present during exposure to the stressor to enhance subsequent analgesia.

Because the benzodiazepines have both anxiolytic and sedative properties and because the latter, but not the former, habituate with chronic dosage, the effects of long-term DZP administration were examined in Exp II. Ten naive rats were injected daily with 2 mg/kg DZP for 20 days. They were then exposed to conditions 1, 4, 5 and 6 as in Exp I. The results in the chronically dosed, DZP tolerant, animals were the same as in the acutely dosed rats.

Exp III examined the effects of DZP upon thermoregulation. Ten chronically dosed rats and nine DZP-naive rats were exposed to four conditions: DZP plus swim 30 mins later; DZP alone; vehicle plus swim; vehicle alone. Core temperatures were monitored before and 15, 30, 45, 60 and 90 mins postinjection. In both chronic and acute dosage, DZP was correlated with a larger temperature drop and a more prolonged course of recovery. Hence DZP may potentiate cold-water swim stress analgesia by altering the subject's experience of, or recovery from, the stressor, rather than by affecting the analgesic process triggered by exposure to stress.

174.10 CHARACTERIZATION OF STRESS-RELATED RESPONSES IN THE MONOSODIUM GLUTAMATE TREATED RAT. D. Badillo-Martinez, N. Nicotera, P. Butler, A.L. Kirchgessner, E. Sperber, and R.J. Bodnar. Dept. of Psychology, Queens College, C.U.N.Y., Flushing, NY 11367.

Medial-basal hypothalamic damage following neonatal administration of monosodium glutamate (MSG) produces analgesic deficits, suggesting that this brain region may be part of an intrinsic pain-inhibitory system. Alternatively, such destruction may interfere with the processing of the stressful consequences of a given stimulus condition. To distinguish between these competing hypotheses, neonatal rats received either a high dose (HMSG: 2 g/kg on days 2 & 4: 4 g/kg on days 6, 8 & 10), a low dose (LMSG: 1 g/kg on days 2 & 4; 2 g/kg on days 6, 8 & 10) or a saline vehicle (SAL). Beginning at 80 days of age, flinch-jump thresholds, thermoregulatory and activity levels were determined following a 2°C swim for 3.5 min (CWS). Pain thresholds, hyperphagia and activity levels were also determined following selected doses of 2-deoxy-D-glucose (2DG). While all groups displayed significant CWS analgesia, the HMSG group showed significantly attenuated effects. Moreover, the HMSG group displayed initial 2DG analgesia at lower doses, but the magnitude of 2DG analgesia across doses was greater in SAL rats. While pre-swim thermoregulation was similar across groups, CWS hypothermia was more pronounced in SAL and LMSG groups (12-14°C) than in HMSG rats (8°C). Furthermore, hypothermia persisted over 1 h in SAL and LMSG rats, but only 0.5 h in HMSG rats, indicating that CWS analgesic deficits of HMSG rats co-vary with its inability to thermoregulate properly following stress. Since CWS induced hypoactivity similarly across groups, this measure failed to co-vary with hypothermic and analgesic deficits. Hyperphagia occurred from 1-5 h after 2DG (650 mg/kg) in SAL rats, but was delayed in LMSG (hours 3-5) and HMSG (hour 5) rats. Hyperphagia was delayed following a 1200 mg/kg 2DG dose in SAL and LMSG rats and absent in HMSG rats, indicating further co-variance between analgesic and hyperphagic deficits. 2DG hypoactivity failed to dissociate the groups. These data suggest that the analgesic deficits following HMSG may be due to an inability to process normally the stressful consequences following exposure to a given stimulus and then subsequently dispay compensatory responses elicited by those stressful consequences. Supported by NIH GRSG 5-S05-RR-07064.

174.11 THE EFFECTS OF ADRENALECTOMY AND DEXAMETHASONE ON THE ANTIOCICEPTIVE EFFECTS OF PHYSOSTIGMINE USING THE RAT TAIL FLICK TEST. J.A. Romano, J.M. King. US Army Medical Research Institute for Chemical Defense, Aberdeen Proving Ground, MD 21010.

Physostigmine, a cholinesterase inhibitor, has been shown to produce antinociceptive responses on the tail flick, flinch jump, hot plate, and tail pinch tests. Certain authors have considered these effects to be mediated by the pituitary-adrenal axis, implying parallels between physostigmine-and stress-produced antinociception. Stress produced analgesia has been shown to be potentiated by adrenalectomy (Pharmacol. Biochem. Behav., 1982, 16:403) and attenuated by dexamethasone (Trans. Amer. Soc. Neurochem, 1981, 12:89). The present study addressed the hypotheses that physostigmine antinociception may be potentiated by adrenalectomy and attenuated by dexamethasone pretreatment.

Seventy adult male Sprague Dawley rats weighing between 225 and 370g were treated on the rat tail flick test (TF). An automated TF apparatus was used with a stimulus temperature of 69.1°C at focal point. Cutoff time for TF latencies was 15 seconds. Animals were tested on two occasions five days apart. Three pre-and three post drug trials were used on each occasion. In a first experiment sham operated animals who received 0.32mg/kg physostigmine salicylate (i.p.) showed elevation of TF post-drug scores on Day 1 followed by no elevation of post-drug TF scores Day 6. On the other hand adrenalectomized animals receiving 0.32mg/kg physostigmine showed statistically significant elevations in post-drug latencies even on Day 6, the second occasion of TF testing.

In a second experiment 0.65mg/kg physostigmine was given to intact animals on two occasions five days apart. These animals also received the synthetic glucocorticoid dexamethasone sodium phosphate (500µg/kg i.p. on Days 4 and 5 and 200µg/kg on Day 6 two hours prior to injection of physostigmine) or saline-control injections. In dexamethasone-treated animals the antinociceptive effect of 0.65mg/kg physostigmine on Day 6 was reduced to nearly one-half of Day 1 effect. On the other hand control animals receiving saline injections in place of dexamethasone did not manifest any reduction of the physostigmine antinociceptive effect. The elevation of the antinociceptive effects of physostigmine by adrenalectomy and the reduction of such effects following dexamethasone suggests that these effects may be mediated through hypothalamic-pituitary-adrenal mechanisms.

174.12 REGIONAL AND LATERAL SPECIFICITY OF BLOOD AND URINE FACTORS INHIBITING FLEXOR WITHDRAWAL REFLEXES IN UNANESTHETIZED RABBITS. A.E.U. Edisen, Y.K. Liu,* W. McMunn,* J. Salinas,* D. Quick,* B. Chandler,* L. Trevino* and R. Wayne*. Div. AH&LS, Univ. of Texas at San Antonio, Texas 78285.

Unanesthetized New Zealand albino rabbits, lightly restrained and in the prone position, were given electroacupuncture stimulation through needle electrodes in acupuncture points Bladder 49 (in the hip region) and Stomach 36 (motor point of the Tibialis Anterior muscle) in one hind limb. Stimulation by biphasic pulses, 0.3 msec. in duration, 2-4 Hz, and ranging from 1.5 to 10 Volts, for 45 to 90 minutes produced a region of insensitivity to pin pricks on the dorsal and lateral surface of the thigh, ipsilateral to the side of stimulation. This analgesic effect was confined to the dorsal and lateral thigh, sometimes extending to upper calf, but not to the ankle, plantar surface of the foot, back, sides or forelimbs. The animals were then sacrificed, blood drained into beakers containing heparin or citrate solution, centrifuged for 5 min. at 2500 rpm at 0°C to prepare plasma. Whole blood was allowed to clot to prepare serum and urine was expressed from the bladders of these donor animals. Unanesthetized naive rabbits with brisk active flexor withdrawal responses of both hind limbs were used as recipients for intravenous infusion of plasma, serum or urine through the marginal ear vein. Doses of these substances ranged from 3-10 ml/Kg and were well tolerated at the temperatures used, 0°C, 10°C, 20°C and 37°C. The pH of urine was 8.5. Analgesia, as indicated by decrease and eventual loss of flexor reflexes to strong pin pricks, appeared in the dorsal and lateral thigh region, sometimes in the upper calf, but not in other regions, within 15 to 75 minutes after infusion and was confined to the same side of the body, left or right, as that of the donor animal. In some recipients, a similar region of analgesia appeared 1.5 to 2 hours later in the contralateral limb. Analgesia persisted up to 2.5 days, and in one case, up to 5 days. These results indicate that substances produced by peripheral nerve stimulation, acupuncture, possess regional and lateral specificity of inhibitory action on flexor reflexes elicited by mechanical stimulation.

174.13 PAIN SENSITIVITY AND PLASMA ENDOCRINE LEVELS IN MAN FOLLOWING LONG-DISTANCE RUNNING: EFFECTS OF NALOXONE. M. Glusman, M.N. Janal*, E.W.D. Colt* and W.C. Clark. Dept. of Psychiatry, Columbia Univ. and NYS Psychiatric Inst., New York, NY 10032

Twelve long-distance runners (mean weekly mileage = 39.85) served as subjects in an investigation of stress-induced analgesia in man and its response to naloxone. Subjects were tested, in a randomly assigned counterbalanced order, on the ischemic pain test for a maximum of 15 min, the cold pressor test for a maximum of 3 min, and a thermal heat test with stimulus intensities of 0, 40, 60, 330, 350, 380 and 400 mcal/sec/sq cm and 3 sec duration. Responses to each of the three tests used an appropriate category rating scale of subjective intensity ranging from nothing to severe pain. Blood was drawn for determination of plasma levels of beta-endorphin (BE) like immunoreactive material, i.e. beta-endorphin and beta-LPH, human growth hormone (HGH), ACTH and prolactin (PRL). Each of these procedures was undertaken before and after a 6 mile run completed in approximately 35 min. Subjects participated on two occasions in a double-blind, counterbalanced design: on one day they received two intravenous injections of 0.8 mg naloxone at 15 min intervals following the run; on the other day, two equal volume injections of normal saline.

Results: Sensory decision theory analysis of the thermal test data showed decreased discriminability, P(A), following the saline control run ($p < .05$), a result consistent with an analgesic effect. This response was partially attenuated by naloxone. Response bias, B, was unaffected. The cold-pressor and ischemic pain tests suggested decreased sensitivity to pain following the saline run, but the results did not reach statistical significance. Naloxone tended to produce divergent results on the post-run cold-pressor and ischemic pain tests, enhancing pain sensitivity in the former and decreasing it in the latter; but again the results did not attain statistical significance.

Plasma levels of HGH, PRL, ACTH and BE were substantially increased by the 6 mile run. HGH was increased 15-fold over the resting level, PRL 5-fold, ACTH 3-fold and BE 2-fold.

The results suggest that long-distance running produces stress-induced analgesia in man. The effect of naloxone was not clear-cut, varying with the pain test used.

(Supported by PHS Grants MH 15174 and GM 26461)

175.1 STIMULATION INDUCED INGESTION IN NEONATAL RATS, Timothy H. Moran*, Gary J. Schwartz* and Elliott M. Blass. Department of Psychology, Johns Hopkins University, Baltimore, Maryland 21218.

Three, 6, 10 and 15-day-old rat pups received 500msec pulse trains of electrical stimulation to the medial forebrain bundle (MFB) at the level of the lateral hypothalamus in the presence of milk. Pups receiving stimulation ingested more milk than littermate controls during the half hour test at 3, 6, and 10 days of age. Day 15 pups did not show this response to stimulation. Stimulated pups were more active than controls through Day 10 and exhibited mouthing, licking, gaping, stretch and even lordosis responses at younger ages. Pups' responses to stimulation became more directed to ingestion with increasing age. The responses to stimulation in the presence of milk were compared to those in an environment without goal objects. Milk augments the activation of stimulation, increasing the incidence of all behaviors in 3-day-old pups, and channels the behavior of older pups into ingestion. Behaviors incompatible with ingestion such as lordosis are selectively inhibited in responses to stimulation in the presence of milk in Day 10 pups. These results suggest a high degree of behavioral organization in neonatal rats that becomes more goal directed with age.

175.2 LONGTERM RETENTION OF CLASSICALLY CONDITIONED RESPONDING IN NEONATAL RATS. Ingrid B. Johanson and W. G. Hall. NC Div. of Mental Health Research Section, Raleigh, NC 27611.

Deprived infant rats as young as 1 day of age respond to oral milk infusions with an impressive behavioral activation, characterized by mouthing, probing, and vigorous locomotion. Components of this behavioral activation can come to be elicited by a neutral odor cue that has been repeatedly paired with milk infusions. The purpose of the present study was to assess long-term retention of such classically-conditioned responding.

Litters of 1-, 3-, and 6-day-old rats were removed from their mothers 24 hr before training. From each litter (n = 9-10 at each age), 3 pups were given 15 pairings of cedar odor with milk (C/M;10 sec of cedar with a milk infusion the last 5 sec; 4 min ITI); 3 pups received milk infusions prior to cedar exposure (M/C); and 3 received cedar exposure only (C). Thus each litter consisted of 3 triplets (C/M, M/C, and C), and each triplet was assessed for retention at 1 of 3 retention intervals. Ten min after training, pups were given 5 10-sec exposures to cedar odor, 2 min apart. During these "CS only" trials, activity, mouthing and probing were scored in the minute following each odor exposure. Pups were then returned to their mother and were left undisturbed until they were deprived 24 hr prior to testing for retention (5 "CS only" trials). For 1-day-olds, retention was tested 1, 2, or 3 days following training; for 3- and 6-day-olds, retention was tested 3, 6, or 9 days later.

Pups conditioned at 1 day of age mouthed and probed more than controls 1 day following training, although there was no evidence of retention 2 or 3 days after training. However, pups (n = 7) that were tested 1 day after training and again at 3 days showed conditioned activity and mouthing 3 days after training. That is, exposure to the CS 1 day after training maintained the effects of training for up to 3 days.

Pups that were trained at older ages retained the effects of conditioning for even longer periods of time. Pups conditioned at 3 days of age were more active and mouthed and probed more than controls 3 days after training, and they probed more than controls 6 days later. There was no evidence of retention 9 days after training. Similarly, pups trained at 6 days of age retained both conditioned mouthing and probing for up to 6 days, and retained conditioned mouthing up to 9 days after the original training. The pattern of responding that occurred in tests of retention was in general typical of the pup's age at retention testing, rather than its age at training.

In summary, these findings indicate substantial long-term retention of a brief appetitive learning experience that occurred in the first few days after birth.

175.3 DIETARY COMPONENT SELECTION BY GENETICALLY OBESE AND LEAN ZUCKER RATS: THE EFFECT OF ADRENALECTOMY. T.W. Castonguay* and J.S. Stern* (Spon: V. Mendel). Nutrition Department and Food Intake Laboratory, Univ. Ca., Davis, Davis, CA 95616.

Adult obese Zucker rats typically eat more than their lean littermates. Although several theories have been proposed to account for this hyperphagia, little is known about how obese rats control the quality of the foods they ingest. Yukimura and Bray (Endocrin. Res. Commun. 5:189-198, 1978) have shown that adrenalectomy can limit overeating and weight gain of the obese rat. The purpose of the present experiment was to determine if adrenalectomy would alter selection patterns of 10 week old obese and lean Zucker rats when they were provided with three macronutrient sources: a protein source, a carbohydrate source and a fat source.

After a seven day baseline period, half of the obese and lean female rats were given bilateral adrenalectomies under Metofane anaesthesia. The remaining half were given sham adrenalectomies. All four groups of rats were then allowed to select their own diets for an additional nine days.

During the baseline period, obese rats ate 20% more calories per day than lean rats. After adrenalectomy, lean adrenalectomized rats and both sham groups did not alter either caloric intake or dietary selection patterns. By contrast, caloric intake of obese adrenalectomized rats was reduced to 50% of its preoperative level. Although there was a tendency for the obese rats to reduce the intake of all three food sources, only the reduction in dietary fat was statistically significant. The obese sham-group selected 28% (5.0g) of their calories as carbohydrate, 30% (5.2g) as protein and 43% (3.3g) as fat. Obese adrenalectomized rats composed diets that were 28% (4.5g) carbohydrate, 34% (4.3g) protein and 38% (1.7g) fat. These differences in percentage dietary composition were not statistically significant. The obese sham-group selected a diet that was significantly higher in fat than the diet selected by the lean adrenalectomized group.

The results from this study suggest that the reduction in the intake of a composite diet may be an adaptation that is more specifically focused on the intake of one dietary fraction, i.e. dietary fat. These results are consistent with the suggestion that beta endorphin may be selectively increasing dietary fat intake in the obese rat, and that an adrenalectomy-induced rise in ACTH may have reduced endorphin activity, and thereby reduced fat intake of the obese rat.

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175.4 COMPETITION BETWEEN FOOD AND BRAIN STIMULATION REWARD: A TEST OF THE REWARD HYPOTHESIS. R.A. Frank. Dept. of Psychology, Univ. of Cincinnati, Cincinnati, OH 45221.

It has been demonstrated that food intake can be dramatically reduced in some rats when brain stimulation reward (BSR) and food are placed in competition during short daily periods. This phenomenon, referred to as self-deprivation, can be observed despite food deprivation severe enough to be life threatening. Two hypotheses have been proposed as explanations for self-deprivation. According to the hunger reduction hypothesis, BSR reduces hunger and thereby decreases food intake. The reward hypothesis of self-deprivation explains reductions in food intake as the result of the greater reward value of BSR as compared to food. Although there have been several convincing demonstrations that BSR does not reduce hunger (e.g., Stutz & Rossi, Physiol. Psychol., 6:204, 1978), no direct test of the reward hypothesis has been attempted.

In order to generate data for a test of the reward hypothesis, rats were implanted with two bipolar stimulating electrodes aimed bilaterally at the ventral tegmental area. Self-deprivation tests were subsequently run for each electrode (left and right) at 40 and 60 uA. Once self-deprivation data had been collected for each electrode and current intensity, animals were given the choice between different pairs of electrode/current combinations in order to determine preferences among the combinations used in the self-deprivation test. According to the reward hypothesis, the electrode/intensity combination that is most preferred should produce the greatest self-deprivation while the least preferred combination should produce the least self-deprivation. Therefore, across all combinations of electrodes and intensities, increasing preferences should be associated with increasing self-deprivation.

The findings provide strong support for the reward hypothesis of self-deprivation. Eighty-eight percent of the predictions of preference that could be made on the basis of the self-deprivation data proved to be correct. Quantitative analyses of the data indicated that the degree of self-deprivation found for a particular electrode/current combination was a good predictor of percent choices for that same combination during a preference test.

175.5 COMPARISON OF TWO METHODS OF ESTIMATING THRESHOLDS OF INTRACRANIAL SELF-STIMULATION. G. Fouriezos and E. Nawiesniak*. BSR Lab., School of Psychology, University of Ottawa, Ottawa, Ontario. K1N 6N5.

Two methods for rapid determination of brain self-stimulation 'thresholds' were compared. The auto-titration method rewards lever-pressing with trains of lateral hypothalamic stimulation that progressively decrease in pulse frequency. Rats may restore the original frequency by depressing a second lever and the point at which they reset is deemed to be the auto-titration 'threshold' (AT). The second method offers the same descending sequence but resets are under timer control; the frequency at which rats cease to earn the stimulation is taken as the timed 'threshold' (TT). Five rats in a within-subjects design were tested in both paradigms. Both AT and TT were stable over time but AT's were consistently higher, indicating that resets were made at values that rats continued to self-administer. Doubling the frequencies in the sequence caused AT's to rise whereas TT's remained anchored at control levels. Changing only the initial frequency by 1 or 2 steps above and below the usual starting value caused AT's to rise and fall respectively whereas TT's were unaffected by this challenge. These results show that resetting behavior in autotitration does not occur at an absolute frequency but instead occurs at some value relative to the level of stimulation available immediately following a reset. The timed method was free of this problem. Both paradigms were tested for their ability to detect amphetamine's threshold-lowering effect. Rats reset at frequencies that were 90, 95, and 102% of control levels following 0.5 mg/kg ip of d-amphetamine sulfate in three tests separated by two days each. The timed method yielded 'threshold' estimates that were 68, 68, and 71% of control performance in identical tests. Together, these experiments demonstrated that the autotitration method does not measure thresholds nor does it properly detect a drug-induced change in self-stimulation threshold. A simple modification of existing implementations of autotitration, that of removing control over resets from the rats' paws, rectifies the problems.
(Supported by Ontario Mental Health Foundation #808.)

175.6 PULSE INTENSITY versus PULSE FREQUENCY MANIPULATIONS IN BRAIN STIMULATION STUDIES. E. Miliaressis, P. P. Rompré* and A. Durivage*. Ecole de Psychologie, Université d'Ottawa, Ottawa, Canada, K1N 6N5.

Self-stimulation, exploratory activity and circling were elicited in the rat by electrical stimulation (0.1 msec in duration cathodal pulses) of the median forebrain bundle and raphe region respectively.

In order to maintain a constant behavioral output, increments in pulse intensity (I) were compensated for by increments in pulse period (P). The large majority of I/P curves were found to fit power functions with index of determination superior to 0.95 and exponents ranging from 1.4 to 0.1.

Subsequent testing with the use of moveable electrodes showed that the value of the exponent depends on the position of the electrode towards the behaviorally-relevant structure. In general, electrodes located outside the target structure generate exponents superior to 1. With electrodes within the structure, the value of the exponent may vary from 1.0 to 0.1 depending on the particular shape and dimension of the structure. In addition, alteration of the stimulated field by electrolytic lesions was found to reverse the value of the exponent from <1 to >1.

A geometric model which accounts satisfactorily for most of the I/P data was proposed. Non-geometric factors were also examined. It was found that factors such as the activation of behaviorally irrelevant elements may play an important role in the determination of the shape of I/P functions.

175.7 TEMPORAL INTEGRATION IN SELF-STIMULATION: A PARADOX L. D. Sax and C. R. Gallistel. Lab. of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104.

We present new findings regarding the temporal integrating characteristics of the substrate underlying self-stimulation in the rat, using a paradigm in which we determine the strength of stimulation required to produce a given level of reward, as a function of the interval separating two short bursts of stimulation. The findings are inconsistent with the leaky-integrator model of postsynaptic temporal integration suggested by Gallistel (J. Comp. Phys. Psych., 92:977, 1978) to explain his data on the strength of stimulation required at different train durations. They are also inconsistent with the adaptation model of Deutsch et al. (Beh. Bio., 29:359, 1980). The data favor a model in which a supra-threshold component of the reward signal is integrated perfectly (without leakage over time) to produce a given level of reinforcement. The linearly increasing charge-duration function (Huston et al., Beh. Bio., 7:383, 1972; and Gallistel, 1978) is also explained by this model. However, the model cannot explain the manner in which the charge-duration function changes as one varies the criterial performance used to derive it. The resolution of this paradox may deepen our understanding of the kind of process by which the transient neural signal generated by the stimulation is converted to an enduring reinforcement effect.

175.8 BRAIN STIMULATION REWARD IN THE LATERAL HYPOTHALAMIC MEDIAL FOREBRAIN BUNDLE: MAPPING OF BOUNDARIES AND HOMOGENEITY. A. Gratton and R. A. Wise. Center for Research on Drug Dependence, Department of Psychology, Concordia University, Montreal, Canada H3G 1M8.

It is well established that electrical stimulation of the lateral hypothalamic medial forebrain bundle is strongly rewarding to the rat. The present study was designed to determine the boundaries and relative sensitivity of the reward substrate in different portions of this region, using a moveable electrode to test multiple sites in vertical penetrations within each animal. Twenty-four male Sprague-Dawley rats were implanted with moveable monopolar stainless steel electrodes (1). Lateral coordinates varied from 0.5 to 3.2 mm from the midline and anterior coordinates were fixed at -0.8 mm from the Bregma (incisor bar elevated 5.0 mm above the interaural line). Rate-intensity determinations of brain stimulation reward were made at 0.25 mm intervals throughout the dorsal-ventral extent of the bundle.

Reward sites were found to extend from the ventral portion of the zona incerta to the base of the brain and from the fornix to the medial edge of the internal capsule. Although more medial placements also supported self-stimulation, this stimulation also produced signs of aversion. Stimulation within and near the internal capsule produced forced movements. The dorsal boundary of the positive region was abrupt; small electrode movements here produced major changes in reward threshold while similar movements had only minor consequences at more ventral sites. Threshold generally increased, though only slightly, as electrodes were lowered to the base of the brain. Within the boundaries of the system changes in stimulation current produced reasonably linear changes in response rate within the range of stimulation intensities from 10-50 μa (sine wave current). There was no systematic difference between medial and lateral thresholds. The MFB substrate for BSR can thus be characterized as dispersed throughout the entire cross-sectional area of the MFB, with relatively homogeneous distribution, but having a slightly lower sensitivity (reflecting, most likely, a slightly lower fiber density) in the more ventral portions of the bundle.

1. Wise, R. A. Physiol. Behav. 1976, 16, 105-106.

Supported by the National Institute on Drug Abuse (DA 01720).

175.9 FASTER ACQUISITION OF MEDIAL PREFRONTAL CORTEX SELF-STIMULATION FOLLOWING PRE-TRAINING STIMULATION OF THE DORSAL PONTINE AREA. A. Robertson*, A. Laferriere* and P.M. Milner*.(SPON: K.Franklin). Dept. of Psychology, McGill University, Montreal, Quebec, Canada, H3A 1B1.

Intracranial self-stimulation (SS) of the medial prefrontal cortex (MC) in rats is acquired slowly, over a period of days. It has been demonstrated that prior non-contingent stimulation of the MC for several days dramatically shortens the number of days it later takes animals to start self-stimulating (Corbett et al., Physiol. Behav., 28, 531: 1982). These data suggest that stimulation-produced reward in the MC develops as a result of repeated exposure to the stimulation.

We have been examining whether we can get the same facilitatory effect of pre-training non-contingent stimulation on acquisition of SS of the MC by giving pre-training stimulation to other points in the brain anatomically related to the MC. In the present experiment, we examined the role of the dorsal pontine area and caudal midbrain (DT). Two groups of rats, each similarly implanted with a MC electrode, were used. One group (n=13) received pre-training stimulation through ipsilateral DT electrodes for nine days, 20 min/day, delivered at a rate of one 0.5 sec train of sine wave stimulation (30-40 uA RMS) every 64 sec. The electrodes were aimed at the area of the mesencephalic and motor nuclei of the trigeminal. The second (control) group (n=11) were treated identically, but did not receive pre-training stimulation. Following this phase, the speed of acquisition of SS of the MC was measured. Each rat was placed for 20 min/day in a box equipped with a response lever delivering 0.5 sec trains of sine wave stimulation (40 μA). No shaping procedures were used. The dependent variable was the no. of days it took a rat to reach a minimum of 100 responses/20 min. Control rats took an average of 6.1 (± S.E.M. .97) days to reach criterion. In contrast, rats which received pre-training stimulation of the DT took only 3.3 ±.89 days ($U = 27.5$, $p < .01$).

Interestingly, the DT group showed the same speed of acquisition as a group of animals (run in a previous expt.) which received pre-training stimulation through electrodes implanted into the sulcal part of the prefrontal cortex (Robertson et al, Physiol. Behav., 28, 1982, in press). Both of these groups took only slightly longer to reach criterion than rats who received pre-training stimulation of the MC itself. Similar pre-training stimulation delivered to the lateral hypothalamic and ventral tegmental areas has been shown to be without effect. Taken together, these data suggest that certain elements within the dorsal pontine area and the sulcal cortex form parts, perhaps interdependent parts, of a neural system involved in the development of the SS and the MC.

175.10 FRONTAL CORTEX SELF-STIMULATION: EVIDENCE FOR INDEPENDENT SUBSTRATES WITHIN AREAS 32 AND 24. L.R. Silva,* J.A. Vogel* and D. Corbett.* Dept. of Psych. and Soc. Rel., Harvard Univ. Cambridge, MA. 02138

Intracranial self-stimulation (ICSS) mapping studies have shown positive sites extending from the rostral medial cortex (area 32) into the cingulate cortex (area 24). It is not known whether the ICSS from these two regions of cortex has similar properties or whether it is mediated by a common neural system. Recently, it has been shown (Corbett et al., Physiol. & Behav., 1982, in press) that the ICSS from area 32 depends upon a system of corticocortical fibers that run laterally towards the sulcal cortices. In view of these findings, it seemed possible that ICSS along the medial cortex might also depend upon a corticocortical pathway, such as the cingulate bundle.

To test this hypothesis we first mapped the medial cortex for ICSS and determined the refractory periods associated with ICSS at different cortical sites. In a second experiment we looked for evidence of functional connectivity between sites in area 32 and 24 as determined by the collision technique (Shizgal et al., J.C.P.P., 94:227, 1980).

The 20 rats used in experiment 1 were implanted with monopolar stimulating electrodes aimed along the medial cortex. Each rat was trained to press a lever for a 500 msec train of 0.1 msec cathodal pulses delivered at 100 Hz. Following response stabilization, rate-intensity data were collected.

Three rats were used in the refractory period and collision experiments. Each rat was implanted with an electrode in area 32 and another electrode in area 24. Pulse pairs were delivered at various C-T intervals to each electrode. Similar pulse pairs were used in the collision tests except that the C pulse was delivered to one electrode while the T pulse was delivered to the other electrode. In both experiments, pulse effectiveness ratios were determined.

ICSS was obtained from area 32 well into the cingulate cortex (area 24). The rate-intensity curves at sites in these areas were similar; low rates with little change in response to increments in current intensity. Refractory periods were similar at ICSS sites in these regions as well, ranging from 4.0-6.3 msec. Finally, there was no evidence for collision between sites in area 32 and 24.

These findings suggest that the systems subserving ICSS within the medial cortex (areas 24 & 32) are similar but independent. Thus, if the output pathways at medial cortical ICSS sites travel within the cingulate bundle, they do so only for relatively short distances.

175.11 PARADOXICAL REINFORCING PROPERTIES OF APOMORPHINE: EFFECTS OF NUCLEUS ACCUMBENS AND AREA POSTREMA LESIONS. N.R. Swerdlow*, D. van der Kooy and G.F. Koob, Behav. Neurobiol. Lab, Salk Institute, San Diego, CA 92138 and Neurobiol. Res. Group, Dept. of Anatomy, Univ. of Toronto, Toronto, Canada M5S 1A8

Many psychoactive drugs have paradoxical reinforcing properties, producing positive reinforcing effects in some paradigms and aversive effects in others. A major step in unravelling these paradoxical properties would be the determination of different brain sites where the drugs act to produce their aversive as opposed to positive reinforcing effects. The present experiment tested the hypothesis that the area postrema is the substrate for apomorphine's aversive effects and the nucleus accumbens dopamine system is the important site of action for the drug's positive reinforcing effects. Apomorphine (.01-10.0 mg/kg, subcutaneously) paradoxically produced both dose-dependent aversive and positive reinforcing effects, as measured in conditioned taste aversion and place preference paradigms, respectively. The conditioned taste aversions produced by apomorphine were not modified in rats with bilateral 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens (producing 92% depletion of dopamine in the nucleus accumbens) nor in rats with thermal lesions of the area postrema. Both types of lesions were behaviorally verified as effective in other paradigms; the 6-OHDA lesions potentiated the facilitatory effects of apomorphine on locomotor activity in photocell cages and the area postrema lesions attenuated the conditioned taste aversions to a novel flavor paired with scopolamine methyl nitrate (1.0 mg/kg, intraperitoneally). However, 6-OHDA lesions of the nucleus accumbens did clearly potentiate the conditioned place preferences induced by apomorphine. These results suggest that both the positive reinforcing and locomotor effects of apomorphine may partially result from activation of post-synaptic dopamine receptors in the nucleus accumbens. Moreover, the dissociation of apomorphine's aversive and positive reinforcing properties revealed by the 6-OHDA lesions may provide the first step in attempts to pinpoint the different brain sites of action where apomorphine produces its opposite motivational effects.

175.12 THERMOREGULATORY BEHAVIORS IN THE RAT AFTER REPEATED EXPOSURES TO HEAT A B Frol Dept of Psychology, Univ of Minnesota, Minneapolis, Mn 55455

Repeated exposures to a hot environment have been found to influence metabolism, sweating, cardiovascular and other autonomic systems in a variety of species. The relative importance of behavior in thermoregulation suggests heat acclimation may also influence heat loss behaviors. In a warm environment rats increase the frequency of grooming, rearing, extension, and activity. These behaviors and brain temperature were observed in rats during repeated short duration exposures to heat followed by a recovery period in a cooler environment.

Adult, male, Long-Evans, hooded rats were placed in cylindrical cages in an environmental chamber. A re-entrant polyethylene tube (PE 10) was implanted in the lateral preoptic nucleus to allow measurement of brain temperature. A testing session consisted of three consecutive periods: a 40 minute adaptation period, a 60 minute stress period, and a 30 minute recovery period. The ambient temperature during all three periods at a neutral exposure was 25°C. During a hot exposure the adaptation period was 25°C, rapidly raised to 40°C during the stress period, and slowly lowered to 25°C during the recovery period. The acclimation phase of the experiment had twenty test sessions, each session given on separate days. Experimental animals received ten hot and ten neutral exposures roughly alternating, whereas control animals received twenty neutral exposures. The testing phase consisted of two sessions at a neutral exposure followed by two sessions at a hot exposure for all animals. Grooming, rearing and extension were time sampled every twenty seconds and recorded. Activity measured by closure of microswitches under the cage and brain temperature measured by a thermocouple were recorded every other minute. Observation began in the last fifteen minutes of adaptation and continued to the end of the test session. The temporal patterning of heat loss behaviors and changes during the acclimation and test phases of the experiment were examined.

175.13 FEAR IN PREFRONTAL RATS. R. R. Holson* (SPON: G. Clark). Dept. of Psychology, University of Washington, Seattle, WA 98195.

Prefrontal lobotomy was long justified by the lowering of chronic anxiety, worry and even pain produced by surgical damage to human prefrontal cortex. Yet no animal model has to date produced incontrovertibly similar results; hence the effects of prefrontal cortical damage upon fear in rats is of considerable interest.

Adult (110 days mean age) male hooded rats were given multiple electrolytic lesions (tungsten electrodes, 2.5 mA DC, 15 secs per placement) in mesial frontal cortex (MFC). Histological verification confirmed that lesions were centered on area 32, with overlying cortex receiving minimal damage. Preliminary analysis of the wet weight of lesioned brains, as compared to sham-lesioned littermate controls, revealed a non-significant drop of 1.5% in MFC whole brain weight. Following fixation in formol-saline, both sets of brains were dissected, and no significant differences were noted in weight of cerebellum, hippocampus, neocortex outside of frontal poles, or brain remnant.

MFC rats were compared to shams on 5 tasks. Each task had two versions, differing in the degree to which subjects would be expected to perceive them as stressful or frightening. Tasks were: 1. HOARDING. (low aversion - covered runways; high aversion - exposed runways). 2. ONE-WAY AVOIDANCE. (low aversion - go from light to dark; high aversion - go from dark to light). 3. EMERGENCE. (from an enclosed box into an exposed corridor. High aversion - prior foot-shock in room; low aversion - no prior foot-shock). 4. BURROWING. (low aversion - burrow to escape open field; high aversion - exposure to open field in order to burrow). 5. OPEN FIELD ACTIVITY. (low aversion - prior handling; high aversion - no handling). On each task, MFC rats differed significantly from controls under high aversion (less hoarding and burrowing, fewer emergences, increased one-way avoidance errors, enhanced activity), but were not different from control under conditions of lower aversion.

It is concluded that one effect of MFC lesions in rats is to enhance reactivity to aversive/frightening situations, and that this effect may account for some of the classic MFC deficits, including hoarding problems and the avoidance deficit. It is premature to speculate on how this enhanced timidity in MFC rats is related to the reduced anxiety seen in lobotomized humans.

175.14 DOPAMINE IN THE NUCLEUS ACCUMBENS MODULATES CLASSICALLY CONDITIONED DEFENSIVE RESPONSES IN THE CAT. W. Jeffrey Wilson and S. Stefan Soltysik. Mental Retardation Research Center and Dept. of Psychology (W.J.W.), University of California, Los Angeles, CA 90024.

Adult cats, trained to criterion in a classical conditioning paradigm in which a tactile stimulus was paired with a 3.0 mA electric shock to the foot, received injections of dopamine (DA) (20, 80, & 320 µg), haloperidol (.5, 1, & 2 µg), or isotonic saline in 1 µl quantities into the nucleus accumbens via bilaterally implanted cannulae. Compared to sessions in which saline was injected, injection of dopamine reduced by 50% the magnitude of the conditioned leg flexion, without affecting the size of the unconditioned flexion. This effect was apparent for all doses of dopamine. Baseline and conditioned heart rate and respiration rate responses were not significantly affected by dopamine. Respiration amplitude, which consistently decreases during presentation of the CS, was also unaffected by DA, as was the probability of a conditioned vocalization.

Haloperidol (1 µg) had no effect on conditioned or unconditioned flexions or vocalizations, nor did it affect conditioned changes in respiration amplitude. However, this dose of haloperidol did cause an increase in respiration rate late in the CS-US period, and a decrease in heart rate for the same period.

The same doses of DA and haloperidol were tested for their effects on locomotor activity in these same subjects. Neither drug affected locomotor activity in an open field, as measured by number of grids crossed per 5 min interval for 30 min.

The fact that DA had its greatest effect on the conditioned motor response supports the contention that dopaminergic input to the nucleus accumbens is involved in the control of motor activity. That only the conditioned responses, and not the unconditioned responses or locomotor activity, were affected is consistent with the hypothesis that the accumbens might link motivation and motor control (Mogenson, et al., 1980; Stevens, et al., 1974). These results, coupled with those of previous experiments in our laboratory in which electrical stimulation of the accumbens had an inhibitory effect on the same motor responses, suggest that dopaminergic afferents to the accumbens from the ventral tegmental area exert a direct or indirect inhibitory influence on the connections within the accumbens between the emotive and motor systems.

Mogenson, G.J., et al. Progress in Neurobiology, 1980, 14.
Stevens, J.R., et al. Psychopharmacologia, 1974, 39.

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175.15 CAFFEINE: EFFECTS ON HUMAN AGGRESSIVE BEHAVIOR. J.L. Steinberg, D.R. Cherek and J.E. Smith. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Aggressive behavior in human subjects was elicited in a laboratory setting and the effects of placebo and 1, 2, and 4 mg/kg doses of caffeine on that behavior were investigated. Subjects were given the option of pressing three response devices. Pressing button A was maintained by a fixed-ratio 100 schedule of monetary reinforcement, consisting of increments of ten cents indicated on a counter and paid to the subject after the session. We defined as aggressive, pressing button B or switch C which ostensibly delivered aversive stimuli by subtracting ten cents from or delivery of a one-second blast of white noise to another "person". Aggressive behavior, i.e., responding on button B or switch C, was elicited by subtracting money from the research subject which was attributed to another "person". The subtractions of money from the research subject took place automatically at random time points throughout the session. Compared to to the placebo condition, aggressive monetary-subtraction responses (button B) decreased in all subjects following the administration of caffeine. The highest dose of caffeine (4 mg/kg) resulted in the greatest reduction in aggressive monetary-subtraction responses. In general, caffeine resulted in similar suppression of aggression noise-delivery responses (switch C) and increased the number of non-aggressive monetary reinforced responses (push button A). The increase in non-aggressive responding following caffeine, indicates that the suppression of aggressive responding was not due to a non-selective generalized depressant action. These findings with caffeine should not be generalized to situations involving coffee-drinking or drinking other caffeinated beverages because of the presence of substances other than caffeine which might have psychoactive effect.

175.16 CORTICAL LOCALIZATION FOR CONTROL OF AFFECT IN HUMANS. R. G. Robinson, K. L. Kubos*, L. B. Starr* and T. R. Price*. Dept. of Psychiatry and Behavioral Sciences, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205, and Dept. of Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Brain-behavior relationships have traditionally developed along two lines. One view described by Lashley emphasizes the importance of the amount of brain tissue removed in producing behavioral change. This is the mass action theory. The second view is based on the topographical localization of behavior, i.e., behaviors such as language expression, aggression or motor control of the contralateral limb are controlled by a specific cortical area. Mood or affect, however, has not been localized by using either one of these concepts. We have looked for relationships between location of brain injury and mood in patients with single focal CT scan verified lesions of either the left (N=28) or right (N=20) hemisphere in right-handed acute stroke patients without any prior risk factors for psychiatric disorder. Based on analyses of CT scan,patients were divided into anterior and posterior groups in each hemisphere. Patients with left anterior lesions (N=10), were the most depressed group (greater mean scores p<.01) and 60% had major affectve disorder by Research Diagnostic Criteria. Within this group there was a strong correlation (r= -.92) between the severity of depression and the proximity of the lesion to the frontal pole (confirming our earlier findings, Ann. Neurol., 9:447, 1980). Fifty percent of patients with left posterior lesions (N=8) had minor depressions. Patients with right posterior strokes (N=6) were significantly more depressed than patients with right anterior lesions (N=6) (p<.05). Two thirds of patients with right anterior lesions were unduly cheerful but apathetic and indifferent. Correlation between depression score and distance of the lesion from the frontal pole (r=.72) went in the opposite direction from that found in the left hemisphere (i.e., lesions farthest from frontal pole were the most depressed). The finding that depression occurs with left hemisphere lesions and right hemisphere lesions is associated with apathy and indifference confirm the findings of previous investigators (e.g., Gainoti, Cortex, 8:41, 1972). However, the intrahemisphere differences (i.e., depression occurs with left anterior and right posterior lesions and apathy occurs with right anterior lesions) suggests that cortical control of affect may be distributed in a graded fashion across the cortex. This kind of localization has not been described for brain-behavior relationships before. This kind of behavioral localization may be based on the anatomy of the noradrenergic pathways and the fact that anterior lesions produce more disruption of these pathways than posterior lesions (Morrison et al., Science, 205:313, 1979).

176.1 CORTICAL VOLUME ASYMMETRY AND BEHAVIOR IN THE ALBINO RAT. G.F. Sherman* and A.M. Galaburda. Department of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, Ma.

The rat has a functionally asymmetrical brain which can be influenced by early experience (Denenberg et al., Sci., 1978, 201, 1150-1152; Sherman et al., Br. Res., 1980, 192, 61-67). In addition, thickness measurements of certain cortical areas have revealed anatomical asymmetries (Diamond et al., Exp. Neurol., 1981, 71, 261-268). However, the relationship between functional and anatomical asymmetry is unknown. In the present study we investigated this relationship and attempted to make a more accurate assessment of the size of specific architectonic areas.

17 brains were obtained from male and female Purdue-Wistar rats handled in infancy and raised in enriched environments or non-handled and raised in standard lab cages. The volumes of the total isocortex, the somatosensory region, the motor region, and the primary visual area on each side were measured. The architectonic zones were determined by well specified criteria (Galaburda and Sherman, Anat. Rec., 1982, 202, 60A) and their total volume measurements calculated from whole brain serial sections.

The total isocortex on the right side of the brain was significantly larger by 1.1% than the left side (t=3.03, p< .01). This was primarily caused by asymmetry present in males. Overall, 8 brains were symmetrical in total isocortex, while 8 of the remaining 9 brains had larger right hemispheres. The asymmetry in favor of the right hemisphere was negatively correlated (r=-.610, p< .01) with total activity in the open field. Since higher activity in the open field reflects lower emotional reactivity, it appears that a larger right hemisphere is associated with a higher level of emotionality.

In both the visual area and somatosensory zones 11 brains had larger areas on the right side, 5 had larger areas on the left, and 1 brain was symmetrical. Although intriguing this distribution is not significantly different from chance and additional brains are being examined. The motor cortex did not show a consistent asymmetry, although in nonhandled animals an asymmetry in favor of the right hemisphere was positively correlated (r=.608, p< .05) with total rightward turning in the open field.

Further analysis of the data revealed that the somatosensory region made up a greater proportion of the total cortex in handled than in nonhandled animals (t=2.66, p< .02). This did not seem to be due to an absolute difference in total or somatosensory cortex, but to the relationship between the two.

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176.2 ROLE OF STIMULUS POSITIONS IN INTEROCULAR TRANSFER OF LEARNING IN PIGEONS. S.Watanabe Dept. of Psychology, Keio Univ.,Mita,Minato-Ku,Tokyo,Japan

Pigeon shows good interocular transfer of both successive and simultaneous discriminations in operant chamber with the pecking key. On the other hand, jumping stand experiments failed to obtain interocular transfer of learning in the pigeon(Graves & Goodale,1977). Such difference might be caused by difference of training apparatus. However, pigeons did not show transfer of spatial conditional discrimination(Green et al,1978) neither sequential order of pecking(Zeier,1976) in the operant chambers. Stevens and Kirsh(1980) also reported a failure interocular transfer of responding trained on FR schedule when pigeons had not received enough motor training with the tested eye.

In the present experiment pigeons were trained on monocular color discrimination in an operant chamber. Two different frontal panels were used. One had a single pecking key on which color stimuli were presented. The other had a pecking key which was illuminated by a white light and a stimulus key on which color stimuli were presented. The stimulus key located 7 cm above the pecking key. One group of pigeons was trained with the single key panel and the other group was trained with the two-key panel. The training procedure was discrete trial training with the positive reinforcement. After the bird learned the discrimination with one eye,retraining with the other eye was given.

When the stimuli were presented on the pecking key (the single key panel),the pigeons maintained their discriminative behavior with the naive eye. That is, they showed almost complete interocular transfer of learning. On the other hand,when the stimuli were presented on the separated key(the two-key panel) the birds failed to transfer their discriminative behavior from one eye to the other eye.

These results suggests that spatial relation of discriminanda(stimuli) and operandum(pecking key) has an important role in interocular transfer of learning in pigeons.

176.3 DOES THE RELATIONSHIP BETWEEN RESPONSE AND REINFORCER INFLUENCE INTEROCULAR TRANSFER IN CHICKS? Karen E. Gaston,* Thomas Altaffer,* & Jenee Todd* (SPON: C. G. Reiness). Pitzer College, Claremont, CA 91711.

Previous work demonstrated that a monocularly-acquired pattern discrimination does not transfer from trained to untrained eye in chicks key-pecking for heat (Gaston, K. E.: Brain Res., 171:339, 1979). This finding is in contrast to reports of interocular transfer for many other tasks in chicks and, especially, to reports that similar pattern discriminations show good transfer in pigeons. The present experiment is the first of a series designed to examine several methodological differences between the pigeon and chick pattern discrimination studies, in an effort to identify factors which determine the occurrence and the extent of interocular transfer in chicks.

In the typical pigeon experiment, a response to the correct stimulus is reinforced by food, whereas in the earlier chick study a correct peck was rewarded by heat. This may be considered an unnatural or non-adaptive association for birds, in that pecking is not normally related to keeping warm as it is to obtaining food. If the adaptive significance of a learning situation influences the extent of interhemispheric communication, then chicks would be more likely to show interocular transfer of the pattern discrimination if they were pecking to obtain food rather than heat.

To test this prediction, 16 5-20 day old domestic chicks were trained monocularly to perform a simultaneous two-choice visual pattern discrimination (+, Δ or Δ, +) for food reward and were tested first with the other eye for interocular transfer and then again with the first eye for monocular retention of the discrimination. The chicks required somewhat fewer trials and errors to reach the 90% criterion with the second eye than with the first (mean trials = 300 & 193.97; mean errors = 99.12 & 65.25), but these savings were not statistically significant. In contrast, the chicks had excellent retention of the discrimination, as evidenced by large and significant savings on both trials (mean = 101.24) and errors (mean = 14.86) when retested with the first eye. There was some indication that chicks trained first with the right eye required fewer trials and errors to reach criterion during acquisition, but demonstrated less savings on both measures during the interocular transfer test, than chicks trained first with the left eye. These possible differences are presently being examined more fully with additional chicks.

The lack of significant interocular transfer observed here in chicks pecking for food is very similar to the earlier pattern of results obtained using heat reinforcement. This suggests that the relationship between response and reinforcer cannot alone account for the previous failure of interocular transfer.

176.4 CIRCULAR CORTICAL KNIFE CUTS INDUCE ASYMMETRICAL BEHAVIOR IN THE RAT. K.L. Kubos*, G.D. Pearlson and R.G. Robinson. (SPON: S.Lukas) Dept. of Psychiatry and Behavioral Sciences, Johns Hopkins Sch. of Med., Baltimore, MD 21205

Previous studies have shown that NE right cortical lesions (Robinson, R.G. and Stitt, T.G., Brain Res. 213:395, 1981) or focal lesions of right cortical cell bodies (Kubos, K.L. et al., Brain Res., in press) cause spontaneous hyperactivity. In an effort to determine the specific contribution of cortical fibers of passage to the asymmetry phenomenon the following experiment was performed.

300 g. male Sprague Dawley rats were housed in running wheel cages for at least 3 weeks prior to surgery. Preoperative spontaneous baseline activity was established during the final week preceeding surgery. Under chloral hydrate anesthesia a fronto-parietal craniotomy was performed, and a 2 mm diameter cut extending to the internal capsule was stereotaxically made with a rotating micro-knife. Identical unilateral lesions were made in either the left or right lateral cortex. Daily activity was then monitored for the next 30 days. Left-lesioned animals demonstrated an initial 30% decrease in activity followed by a gradual return to preoperative baseline level. Right-lesioned rats, on the other hand, demonstrated an initial increase in activity which peaked at 120% above preoperative baseline levels at day 18 and subsequently fell to 80% by day 30. Both left and right lesioned groups were statistically different from controls as well as from each other.

176.5 BINOCULAR DEPTH PERCEPTION IN CATS FOLLOWING NEONATAL SECTION OF THE CORPUS CALLOSUM. Brian Timney[1], Andrea Elberger[2] and Leslie Vandewater*[1]. [1]Department of Psychology, University of Western Ontario, London, Canada and Departments of Anatomy and Neurobiology, University of Texas Medical School, Houston, Texas.

Recently we reported that neonatal section of the optic chiasm in kittens leads to a relatively minor impairment of binocular depth perception (Timney & Lansdown Soc. Neurosci. Abs. 1981, 7, 674). The results indicated that an indirect pathway, presumably through the corpus callosum, was sufficient to mediate this ability. The present study was designed to establish whether the corpus callosum was necessary for the maintenance of good stereoacuity.

Three kittens underwent section of the posterior half of the corpus callosum within two weeks of birth and a fourth kitten was given a sham operation. The surgery was carried out at the University of Texas then the kittens were shipped to Ontario for testing. The Ontario investigators did not know the experimental condition to which each animal had been assigned, except for an additional kitten which served as a normal control.

Binocular and monocular depth discrimination thresholds were measured using the jumping stand technique, in which the cats were trained to jump from a platform towards the closer of two surfaces placed beneath them. Thresholds were calculated as the smallest separation that could be discriminated with 70% accuracy.

All three of the callosum-sectioned kittens performed indistinguishably from normals. Binocular depth thresholds (approx. 6 min/arc disparity) were much better than monocular, suggesting that the animals had retained their ability to use uniquely binocular cues to depth. In order to determine whether the excellent performance was due to some form of development compensation, the corpus callosum of the normal kitten was sectioned (by F. Lepore, Université de Montréal) at the age of 4½ months. There was no difference between pre- and post-operative thresholds, suggesting that an alternative pathway was subserving the discrimination. However, section of the optic chiasm in the callosum-sectioned cats, which eliminated the possibility of binocular convergence for these animals, led to dramatic deterioration of performance.

The results, in conjunction with those obtained previously, suggest that either the optic chiasm or the corpus callosum provide pathways sufficient to maintain stereoscopic vision, but neither structure seems to be necessary.

176.6 LOCALIZATION OF INTERHEMISPHERIC VISUAL CONNECTIONS IN MACAQUES. C. R. Hamilton, S. B. Tieman, B. A. Vermeire and R. L. Meyer. Division of Biology, Caltech, Pasadena, CA 91125.

The main purpose of these experiments is to establish with reasonable accuracy the location in the cerebral commissures of fibers connecting different regions of visual cortex. If a significant separation of connections from various cortical areas exists, then it is possible to prepare and behaviorally test monkeys with intact interhemispheric connections restricted to a subset of visual areas. A knowledge of the kinds of visual information that can still be transferred between the hemispheres would allow inferences to be drawn about the functions of the visual areas that remained interconnected. A second point of the experiments is to determine the major heterotopic targets of commissural projections from various regions of visual cortex.

Tritiated proline and discrete lesions were placed in several regions of visual cortex in 12 macaques and after one week survival the brains were processed by standard autoradiographic or reduced silver methods. The visual regions studied included the 17/18 border, MT, V4, and IT cortex. The localization of fibers in the splenium of the corpus callosum was quite discrete. Furthermore, there was a continuous progression of fibers in the splenium from postero-ventral to antero-dorsal that corresponded with the cortical order 17/18, V4, MT. Essentially all fibers leaving IT cortex passed through the anterior commissure. All the regions investigated sent fibers to homotopic regions of the opposite hemisphere; these projections usually were patchy. Heterotopic projections were best seen with the proline injections. They were most striking for injections in and around V4 and MT and projected predominantly to contralateral areas "downstream" from the injection site. Overall, these results encourage the continued behavioral study of interhemispheric transfer in monkeys with commissural connections restricted to selected subsets of visual areas.

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176.7 TILT AFTEREFFECTS IN MONKEYS. B. A. Vermeire and C. R. Hamilton. Division of Biology, Caltech, Pasadena, CA 91125.

The tilt aftereffect, the illusion that a vertical line appears tilted after prolonged viewing of an oblique grating, has been extensively studied with human subjects. Recent interest in this illusion has centered on the suggestion that interocular transfer of the aftereffect depends on the integrity of binocular neurons in the visual cortex. This is inferred from the finding that strabismic and amblyopic animals lack binocular neurons in the visual cortex and that people with similar disabilities usually fail to show the expected interocular transfer of the tilt aftereffect. A more direct assessment of the dependence of interocular transfer on binocular neurons and of the visual areas involved in this aftereffect could be made if tilt aftereffects were measurable in experimental animals.

Four monkeys (M. mulatta) were tested for tilt aftereffects using a modified method of constant stimuli. All monkeys had their optic chiasm sectioned so that the magnitude of the aftereffects, if present, would serve as a baseline for subsequent studies of interocular transfer in partially split-brain monkeys. The monkeys were trained to gaze at a high contrast vertical square wave grating projected on a screen. When the grating occasionally dimmed, the monkeys pushed the screen to initiate presentation of a test line oriented (in $1\frac{1}{4}^{\circ}$ steps) between 0° and $\pm 7\frac{1}{2}^{\circ}$. The monkeys were rewarded for pushing again if the line was vertical and for not pushing if the line was tilted. To produce aftereffects, the grating was tilted $\pm 10^{\circ}$. Each monkey, tested binocularly, showed reliable aftereffects of about 1° on each of eight separate sessions. Human subjects using the same apparatus showed aftereffects of about 1.5°; the somewhat smaller aftereffects of the monkeys probably reflect less complete adaptation associated with viewing the grating about 80% of the time. We are now testing partially split-brain monkeys for interocular transfer of these aftereffects. These monkeys have cortical binocularity restricted to the vertical midline of the visual field and to a subset of visual areas.

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176.8 INTERHEMISPHERIC MNEMONIC TRANSFER IN MACACA NEMESTRINA. Robert W. Doty, John A. Gallant* and Jeffrey D. Lewine*. Center for Brain Research, University of Rochester Medical Center, Rochester, New York 14642.

Monkeys with optic chiasm transected via a transsphenoidal approach are trained to identify with one eye and hemisphere photographic "slides", unique for each trial, viewed 10 sec previously by the other. The monkey wears a light-weight mask equipped with shutters to provide selected monocular input. When performance is consistently correct for 90% of the 50-100 daily trials, either the entire corpus callosum (CC) is transected, leaving the anterior commissure (AC); or the AC and rostral CC are cut, leaving only 4-5 mm of the splenium. One each of such cases has now been tested, for >5600 trials each, and performance is indistinguishable. For the first two trials, given 60 days postoperatively, requiring use of AC only, the monkey appeared completely baffled, but then gradually accommodated, failing to respond to only ten of the first 50 trials, and achieving 94% correct for the 40 choices which it did make. Even this slight hesitancy was absent from the postoperative session in the animal performing interhemispheric transfer for the first time with only splenium remaining. Performance with color photographs of complex objects or scenes is distinctly better (85-95% correct) than with pure, objectless color (80-85% correct); but no consistent difference has been found between hemispheres (for intrahemispheric comparison, i.e., same eye) or for direction of transfer.

The intention had been to test also with abstract geometric patterns, using 2-6 stroke Japanese Kanji. The monkeys, however, were unable to attain preoperative performance consistently above chance despite 2200 and 2900 trials of training, even at 0.5-4 sec delay intervals. Since non-Japanese human subjects achieve 100% correct at 10-sec delays without the slightest difficulty, an interesting difference between human and macaque visual mnemonic organization is perhaps manifest here. That the monkeys' difficulty is not attributable to mere absence of color in the Kanji stimuli was shown by their postoperative ability to achieve interhemispheric identification with almost equal facility for two series of the same objects, one in "black and white" (85% correct) versus color (90% correct).

Using a "list" procedure in which response must be withheld upon a second presentation of any "slide" among the 280 viewed in a given session, one monkey with AC and chiasm cut (all CC intact) maintains a 74% correct interhemispheric identification at a mean delay of 26 sec (range 10-50 sec). There is a suggestion of left hemisphere superiority at the longer intervals.

The main conclusion to date is that for interhemispheric identification of color or complex objects after 10-sec delays the AC and CC offer essentially equivalent pathways.

177.1 SENSORY REACTIVITY, HABITUATION, AND ASSOCIATIVE LEARNING IN DECEREBRATE HUMAN INFANTS. G. G. Berntson, A. E. Ronca* and D. S. Tuber*. Lab. Comp. Physiol. Psychol., Ohio State Univ., Columbus, OH. 43212.

Human infants with extensive damage to, or total loss of, the cerebral hemispheres were tested for sensory reactivity, habituation, and associative learning in a conditioned expectancy paradigm employing heart rate measures of the orienting response. Two stimuli were repeatedly presented to the subjects in close temporal contiguity. Interspersed within these conditioning trials were occasional test trials in which the first stimulus was presented and the second stimulus withheld. In 3 of the 6 subjects, cardiac orienting responses (generally decelerator) on the initial trials indicated that the sensory stimuli were functionally processed. Within a few trials, however, these cardiac orienting responses appreciably declined, reflecting the normal process of habituation. A reinstatement of the cardiac orienting response was seen on test trials, in which the first stimulus was presented and the second stimulus withheld. This finding indicates that a stimulus-stimulus association had also been formed. In these cases, the orienting response was seen to the omission of a stimulus, an omission which could be detected only to the extent that a conditioned expectancy or stimulus association had been established.

In three additional infants, no evidence of associative learning was seen. In each case, however, there was minimal reactivity to the sensory stimuli, and when present, this minimal reactivity was not subject to habituation. CAT scans failed to reveal clear differences in the functional level of decerebration in those subjects who showed habituation and associative learning and those who did not. Thus, the autonomic indices employed appear to reflect the functional integrity of the remaining neuraxial levels, rather than the absolute level of decerebration. The present results document the cognitive processes of habituation and associative learning in the virtual absence of the cerebral hemispheres. These findings are in keeping with a growing body of literature detailing the extensive functional capacities of lower levels of the neuraxis. Moreover, the results suggest a method for the evaluation of cognitive processes in severely brain damaged or otherwise impaired infants and children. These studies are currently being extended to a broad population of mentally retarded and developmentally delayed infants and children.

177.2 A BRIEF SCALE FOR RATING BEHAVORIAL DEFICITS IN DEMENTIA James A. Haycox, M.D.

To evaluate and follow the progression of dementia in a Dementia Day Treatment Program, a brief and robust scale for measuring both cognitive and behavioral deficits was needed.

Good scales already existed for measuring cognitive deficits. A scale which can be administered in less than ten minutes has now been developed for rating the relevant behavorial deficits based on observation of precise forms of behavioral deterioration. Eight categories of behavior are rated: (1) language competence, (2) social interaction, (3) attention, (4) spatial orientation, (5) motor performance, (6) bowel and bladder control, (7) eating and nutrition, (8) and dressing-grooming competence. A score of zero in any category signifies full function while six indicates terminal disability. The minimum total score (0) indicates healthy-mindedness and the maximum score is 48. Para-professional raters achieved reproducibility ($r=0.9$) after a minimum of training and high correlation ($r=0.8$) with clinical judgements of patients' status done independently. We have evaluated and followed over 300 patients at risk for dementia. Analysis of our follow-up data demonstrates correlations with autopsy findings, contrasts the findings in several diseases causing dementia and illustrates the progression of dementia when treated in day care and when not treated.

177.3 VERBAL LEARNING AND REMOTE MEMORY FUNCTIONS IN ALZHEIMER-TYPE DEMENTIA. B.A. Thompkins-Ober*, B. Koss, R.P. Friedland, T.F. Budinger*, E. Ganz*, and D.C. Delis*. Dept. Neurology, University of California, Davis, V.A.M.C., Martinez, CA 94553 and Donner Laboratory, University of California, Berkeley.

Eight patients, clinically diagnosed as exhibiting Alzheimer-type dementia participated in a multidisciplinary study including neuropsychological evaluation, x-ray computerized tomography (CT), and positron emission tomography (PET) with 18-fluorodeoxyglucose. The patients (aged 55-75) were classified as either moderately or severely demented based on their Mattis Dementia Scale score. The performances of these two subgroups of four patients each were compared on both verbal learning and remote memory tasks. A Buschke selective reminding procedure was used to evaluate verbal learning abilities. After an initial presentation of the list of words, alternating study and recall trials were administered with presentation on the study trials of only items missed on the previous recall trial. The severe dements recalled fewer total items at the end of learning. However, the proportion of items recalled from long-term store (recalled at least once without representation) out of the total number of items recalled from both long-term and short-term store (without representation) was actually greater for the severe dements. This suggests differential learning strategies between the two groups. The moderate dements are more likely to repeat back the represented words on each trial in order to maximize recall, and therefore are distracted from encoding items into long-term store. In contrast, the severe dements seldom repeat back any of the represented words and instead focus exclusively on a few words from the list, increasing the probability of encoding these words into long-term store. Twenty minute delayed recall of the list showed greater memory loss of previously learned words for the severe dements.

Albert's remote events recall questionnaire yielded a measure of remote verbal memory. The moderate dements recalled many more events and their performance benefited more compared to severe dements when multiple choices were given for each question. This is indicative of a remote memory impairment which for moderate dements, but not for severe dements, can be overcome by the elimination of retrieval requirements.

These memory data will be discussed in relation to cerebral structural and metabolic abnormalities in these patients as documented by x-ray CT and PET.

177.4 COGNITIVE DEFICITS IN MULTIPLE SCLEROSIS. L.M. Nelson*, D.S. Thompson*, R.H. Heaton*, J.S. Burks*, S.H. Walker*. (SPON: L. Minier) Rocky Mountain Multiple Sclerosis Center, Dept. of Neurology, University of Colorado Med. Sch., Denver, CO 80262.

Prior studies of possible cognitive impairment in MS have not accounted for disease activity or made a distinction between clinical subtypes, [i.e. relapsing/remitting (R/R) and chronic/progressive (C/P) MS]. One-hundred consecutive patients with clinically definite MS (57 R/R tested in remission, 43 C/P) and 150 normal controls of similar age and education were given an expanded Halstead-Reitan Battery (HRB) measuring a broad range of cognitive and sensory/motor abilities. Group comparisons were made using analysis of covariance with adjustment for age and duration of disease.

The total MS group was not different from controls on the information and vocabulary subtests of the WAIS, suggesting comparable premorbid intellectual functioning in the MS group.

On the expanded HRB, the C/P group had several mean cognitive scores in the impaired range even on tests which require little or no motor ability (i.e. Category Test, Story and Figure Memory). The strongest differences between controls and C/P patients (as reflected by the size of F-ratios) were obtained with tests requiring complex problem-solving, concept formation, and sequencing. The R/R group tested in remission was not significantly different from the control population on any individual non-motor test. The profile of cognitive deficits among R/R patients was of a very mild nature, and showed no consistent pattern. Data will be presented examining the effects of acute exacerbations and life stressors on cognitive functioning.

The degree of cognitive impairment was related to duration of disease, but not to number of recorded disease exacerbations. The comparison of medicated versus nonmedicated subgroups revealed no significant differences on tests of cognitive abilities, indicating that the increased incidence of cognitive deficits among C/P patients was not due to medication effects.

Blind clinical ratings revealed that 57% of R/R and 74% of C/P patients had impaired cognitive functioning. For the purely cognitive tests of the HRB, a 60% concordance was noted with a clinical mental status exam in predicting cognitive impairment.

The neuropsychological profile is a necessary supplement to the clinical history and mental status exam in determining the presence or absence of cognitive involvement.

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177.5 PRESENCE AS A NEW MODE OF HUMAN ATTENTION, Vinod D. Deshmukh, 3600 Rustic Lane, Jacksonville, Fla 32217

It is proposed that human attention has two fundamentally different modes. The commonly recognized human attention is called 'Mnemic' and the 'New' mode of human attention is called Presence. The word Mnemic means of or related to memory. In Mnemic mode of human attention there is focussing or directing of partial attention to a particular object in the environment. There are habitual cognitive and emotional responses based on memory that constantly interfere during the mnemic mode of human attention. This also creates the observer-observed duality and experience of personal time and change. It creates sustains and adds to the self image. It always limits perceptivity attentional energy, intelligence and feeling of goodness or natural happiness.

The 'New' mode on the other hand is evernew. It is not contaminated by the past memories. It is complete participant attention to the present-now and here. It is free of any habitual cognitive or emotional responses. It is free of self image with no interference with the present human activity. There is no divison as 'I' and the 'rest' or not-I. There is no projection into the future based on the past. It implies tremendous frictionless, conflictless attentional energy, intelligence and a feeling of self-freedom and natural goodness. Since it is completely one with the present-the what is; it is named as the state of Presence.

An hourglass model of observer and the observed is presented to explain the functoning of mnemic and new modes of participant attention. This model and above modes of attention also help to understand and to resolve the problem of Brain-Mind duality in Neuro Sciences.

177.6 THE PROCESSING OF EXPECTED AND UNEXPECTED EVENTS DURING CONDITIONING AND ATTENTION. S. Grossberg, Center for Adaptive Systems, Dept. of Math., Boston Univ., Boston, MA 02215.

Recent models of Pavlovian and instrumental conditioning can be unified to yield a theory wherein many conditioning, cognitive, motivational, psychophysiological, and pharmacological data can be synthesized and predicted(1). Some recent formal models contain internal paradoxes that restrict their predictive power. These paradoxes can be traced to an inadequate formulation of how mechanisms of short term memory and long term memory work together to control the shifting balance between the processing of expected and unexpected events. Once this formulation is strengthened, a unified processing framework is suggested wherein attentional and orienting subsystems coexist in a complementary relationship which controls the adaptive self-organization of internal representations in response to expected and unexpected events. Using this framework, conditioning and attentional constructs can be more directly validated by interdisciplinary paradigms in which seemingly disparate phenomena can be shown to share similar physiological and pharmacological mechanisms. A model of cholinergic-catecholaminergic interactions suggests how drive, reinforcer, and arousal inputs regulate motivational baseline, hysteresis, and rebound with the hippocampus as a final common path. Extinction, conditioned emotional responses, conditioned avoidance responses, secondary conditioning, and inverted U effects also occur. A similar design in sensory and cognitive representations suggests how short term memory reset and attentional resonance occur, and are related to evoked potentials like N200, P300, and CNV. Competitive feedback properties such as pattern matching, contrast enhancement, and normalization of short term memory patterns make possible the hypothesis testing procedures that search for and define new internal representations in response to unexpected events. Long term memory traces regulate adaptive filtering, expectancy learning, conditioned reinforcer learning, incentive motivational learning, and habit learning. When these mechanisms act together, conditioning phenomena like overshadowing, unblocking, latent inhibition, overexpectation, and behavioral contrast emerge.

Reference: Grossberg, S. Psychological Review, Sept. 1982.

Grossberg, S. In J. Cohen, R. Karrer, and P. Tueting (Eds.) Cognition and brain activity. NY:NY Acad. of Sci., 1982.

177.7 MUSICAL THOUGHT DISCLOSES A HIGHLY STABLE PSYCHOBIOLOGIC CLOCK, M.Clynes, B.McMahon* and N.Nettheim* NSW State Conservatorium of Music, Sydney 2000 Australia.

Musical thought has the ability to activate psychobiologic clocks with a long term stability of 1 part in 500 or better[1]. The existence of such clocks presents a theoretical challenge. The unexpected findings also suggest that under proper experimental conditions such a psychobiologic clock could become a sensitive vehicle for the study of highly specific neurohormonal or drug influences and function.

This stability has been demonstrated through:

1. Performances over a number of years of major portions of several minutes duration of musical compositions by single performers as well as by ensembles (including string quartet). Pieces with a regular motoric pattern show greatest stability. For slow pieces stability tends to be less.

2. In experiments involving repeated tapping to silently thought music (each tap period measured to 0.00001 sec.) mean tapping-rate of groups of 1000 taps can be stable to within 1 part in 500 when a musician taps to the beat of a specific known allegro musical theme (e.g.Mozart K467 1st Movt.) thought silently and repeatedly. It was found in a series of experimental runs involving 4000 taps each that under these conditions the stability of the mean tapping rate increases with the number of taps whose duration is being averaged; over a range of groups of 1 to 1000 taps, the standard deviation decreases from 4% for single taps to less than 0.3% for groups of 1000 taps (only a factor of 2 less than the theoretical limit for the signal/noise improvement if the period consisted of a constant plus random noise.)

Other experiments comparing performances thought in real time with actual performances consistently show the thought performance to be slower by several per cent; as tested in 400 performances of professional musicians. (Clynes, in press).

These findings indicate that musical thought can activate highly stable psychobiologic clocks whose stability within the above limits is not affected by inner and outer environmental factors encountered by these musicians under normal working conditions.

1. M.Clynes & J.Walker, Rhythm, Time and Pulse in Music: Neurobiologic Functions, pp.171-215 in Music, Mind and Brain: The Neuropsychology of Music, ed.M.Clynes, Plenum New York, 1982.

177.8 INFORMATION PROCESSING (BASIC DIFFERENCES BETWEEN THE CENTRAL NERVOUS SYSTEM (CNS) AND THE COMPUTER) C.Torda. Res.Dpt.,N.Y.Center PA Train.(Curr.Addr.P.O.B.4866,Stanford Univ.,Calif.,94305).

With the development of artificial intelligence the problem of differences in information processing by the CNS and the computer emerged.Computers exceed human performance by speed, accuracy, consistency and near-perfect logical relationship between input and output. Since computers were created by human skill and intelligence one may expect the performance of the CNS to exceed that of the computer.This problem has been addressed in the current study. The various logic systems employed by the different programming techniques, symbol formation, coding, transmission of the coded information,memory storage and retrieval by the CNS and the computer have been compared.The known CNS mechanisms have been analyzed from the point of view of stimulus detection, transformation of exo- and endogenous energy forms into the form the CNS is able to utilize,the complex and multiple prevalently parallel coding(including their biophysical and biochemical regulation) and the central mechanisms for abstraction,integration,symbol formation,organization, regulation,etc.Through continuously changing oscillatory processes spontaneous interplay of excitatory and inhibitory circuits maintain a near-threshold state in absence of incoming stimuli.This selfgenerated local activity is selfregulated(mainly through the interplay of the glutamate-GABA cycle). Thus local processes may affect the nature of the newly generated codes. Every CNS output results from the interplay of several excitatory and inhibitory processes. By prevalence of one or the other putative neurotransmitter the different states of consciousness exert systemic regulation of stimulus sensitivity of the various networks.Order and some consistency is maintained by organizer, regulatory and decision systems,to name a few:the pace-maker neurons, the left and right hemispheres,time-keeping systems,the cyrcadian rhythms,the viscero-autonomic(VA)system,etc.Each VA process creates not only emotions,but also one nonspecific systemic inhibitory circuit through which nearly all other network activity is affected.The CNS processes are constantly affected by plasticity, the cognitive past and current physiological conditions.CNS memory is complex, multiple,distributed.The content constantly changes with performance and is subject to the quantum mechanical laws for submolecular systems.Any input into the CNS generates several output types,following various goals including preservation of survival of the organism.The final output is selected only partly by the input,and mainly by a decision system.This seems to contribute to the occasionally questionable logic of the CNS response.Human logic thus may appear erratic because human insight into the CNS processes is defective.We lack insight into the nature of the submolecular processes of the CNS, thus many processes appear as automatic and escape our conscious perception, including a potentially existing superior perfect logic system.

177.9 A COMPUTER MODEL FOR LEARNING PROCESSES AND THE ROLE OF CEREBRAL COMMISSURES, P.A.Anninos and P.Argyrakis*. Dept. of physics, University of Crete, Iraklion Crete, Greece.

Artificial neural nets constructed of discrete populations of neurons have been studied through computer simulation (Anninos,P,Kyb. 11,5,1972) in order to verify the potential role played by the great cerebral commissure as was pointed out by R.Sperry (Neurosc. vol 1 714,1967) in his experimental studies.

In our first theoretical study we consider the simulated intact brain consisting of two neural nets constructed with the same statistical parameters ($K^{\pm}$, ϑ, $\mu^{\pm}$, h), but different microscopic structures (Anninos, P.Kybern. 11, 5,1972), and with their interconnections ($\mu_{12}^{\pm}$ and $\mu_{21}^{\pm}$) corresponding to the intact corpus callosum. In addition, to each neural net or simulated hemisphere we consider a steady input with connections to both neural nets in order to simulate a process such as for example the incoming inputs to the two hemispheres from the two eyes. For the range of parameters used we found that these nets may respond in specific manner to specific stimuli, and furthermore whatever memory was transfered to one net was also transfered to the other. (In these studies the response of the system is monitored by the rise of cyclic activity, as descibed elsewhere (Anninos, P. Kyb. 11,5,1972). However, this behavior changed when the two nets were seperated. When the inter-connections were eliminated the response to each net was different for the same stimulus, so that the brain response to a given situation depended upon which net received the triggering stimulus. The situation changes after learning takes place, in which case the response of both nets was the same. (Learning was considered according to the synaptic facilitation rules, Papadopoulos et al, J.Theor. Biol, 80, 505, 1979).

The above findings verify the experimental observations of Sperry's work that the split brain behaves as if it were indeed two separate brains each performing concurrently and simultaneously diametrically opposite tasks.

177.PO Acquired Neurological Stuttering: Report of Two Cases. W. J. Nowack and R. E. Stone*. Departments of Neurology and Otorhinolaryngology (Speech Pathology), Indiana University School of Medicine, Indianapolis, IN 46223

The value of dysphasic disorders in localizing underlying neurological lesions has repeatedly been demonstrated clinicopathologically. There is debate in the literature about whether a less-frequent speech disorder, stuttering acquired in adulthood is associated with a specific central nervous system lesion. In fact, there have been those who have argued that stuttering acquired in adulthood is primarily a hysterical, psychogenic disorder. We have seen two cases at the Indiana University Medical Center which might help clarify the issue.

Case I: A 30 year old white female with no past history of speech disorder suddenly developed stuttering associated with the occurrence of new domestic difficulties. Her electroencephalogram, which had demonstrated left temporal sharp wave activity without spread to the right side at past times when she had had no speech disorder, now showed bitemporal sharp wave discharges. A course of speech therapy designed to improve airflow proved to assist the patient in retraining the motor mechanisms of speech production and to be anxiolytic to the patient. Following resolution of her domestic difficulties, her stuttering disappeared.

Case II: A 55 year old female, who had experienced a left hemispheric stroke, which did not produce stuttering, developed stuttering after a second right hemisphere stroke. Much less tissue was destroyed in the non-dominant (right) hemisphere than in the dominant (left) hemisphere, as demonstrated by CT scan of the head. Following some domestic difficulties, her stuttering worsened markedly. A similar program of anxiolytic speech therapy was begun. Her stuttering was decreased markedly and her communicative ability increased significantly, although some stuttering was still present.

Both cases suggest that stuttering is associated with bihemispheral disease with the greater pathological change in the dominant hemisphere. The two cases further suggest that the patient's emotional state can interact with the organically caused speech deficit to render the stuttering worse. These observations resolve some of the apparent contradictions in previous reports. Speech therapy which is primarily anxiolytic but which does not correct the underlying neuropathology can prove useful in the management of patients with stuttering acquired in adult life.

178.1 FUNCTIONAL RECOVERY OF BEHAVIOR IN ANIMALS FOLLOWING BRAIN TRANSPLANTS, H. Koopowitz, L. Davies*, and C. L. Keenan*. Developmental and Cell Biology, Univ. of Calif., Irvine 92717.

Brains transplanted from one individual flatworm (Notoplana acticola) into the body of a different decerebrate individual establish anatomical connections, within 24 hr, with the peripheral nervous system of the acceptor worm. Recovery of behavior occurs in these preparations as does recovery of function in identified neurones of the brain. Partial reappearance of simple behaviors appears 48 to 72 hr following the transplant and total recovery appears within the first 10 days. Simple behaviors include ditaxic walking, righting and avoidance turning. Usually the behaviors reappeared in the sequence above. More complex behavior such food capture recovers erratically. Some individuals recover within a few days but others require 20 days to recover this behavior. Of 60 transplants attempted, 42 reestablished all four behavior patterns. Even when the donor brain was rotated, functional connections were reestablished. Donor brains were placed in one of four possible orientations: normal, anterior-posterior reversed, dorsoventral inverted, and anterior-posterior reversed with dorsoventral inversion. All experiments were run double blind and all animals were tested for behavioral recovery each day. There were five trials for each behavior tested. Although 15 transplants were performed for each group, there was differential survival. Data is either expressed as means or as individual performances. The latter case was used if recovery was erratic. All animals were fixed and examined histologically to confirm the orientation of the donor brain. Intracellular investigations of vibration responder interneurones were performed in worms which had accepted anterior-posterior reversed brains, 20 days after surgery. These cells had reestablished connections with peripheral sensory neurones. Lucifer fills of these cells indicated that they were able to grow to appropriate regions in the peripheral plexus although this required complex routes.

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178.2 INPUT PROPERTIES OF CLOSER MUSCLE FIBERS IN DIMORPHIC CLAWS OF SNAPPING SHRIMP. DeForest Mellon, Jr. and L. David Smith. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22903

Transformation of claw type in snapping shrimps (Alpheus) is accompanied by an increase in claw closer muscle volume due to specific, renewed growth of individual, pre-existing adult muscle fibers. During transformation, fibers in the closer muscle of the original pincer claw continuously increase their diameter until, in the fully transformed snapper claw, the fibers are four to five times as broad as in their original form. Furthermore, excitatory junction potentials (ejp's) in closer muscle fibers of transformed claws are as large or larger than those exhibited by homologous fibers in non-transformed pincer claws. In theory, during growth fiber input resistance, R_0, varies inversely with the muscle fiber diameter, d, such that $R_0 \sim d^{-3/2}$ as long as sarcoplasmic resistivity, R_i and specific membrane resistance, R_m, remain constant. This relationship holds strictly during growth of identified superficial flexor muscles in the crayfish abdomen. We wished to know whether the input resistance of pincer and snapper closer muscles in snapping shrimps is determined by the theoretical relationship with fiber diameter and, if so, the mechanisms employed during transformation to maintain adequate excitatory synaptic potential amplitude as the target muscle fiber volume enlarges and electrical resistance falls. We used separate microelectrodes to pass current into claw muscle fibers and to record resultant membrane potential charges. Our results show that the mean input resistance of 44 snapper closer fiber was 32 Kohms and that for 25 pincer closer fibers was 151 Kohms, suggesting the input resistance of closer fiber does fall during transformation. The relationship between d and R_0 in these fibers is close to theory. The data from 25 closer fibers, the majority from paired pincer and snapper claws, are best fit by a power function, in which $R_0 \sim d^{-1.59}$. The input resistances of fibers from the two types of claw lie along a continuum, in which size is the obvious independent variable. The possibility that either R_i or R_m also show a consistent relationship with fiber size was examined. Both of these parameters vary widely in muscles of the two claw types, but neither is consistently dependent upon muscle fiber size. We conclude that during pincer-to-snapper claw transformation, R_0 of closer muscle fiber increases as a consequence of geometrical factors alone. Since ejp's of snapper closer muscle fiber are larger than corresponding activity in pincer claws, excitatory synaptic currents must increase during transformation. Statistical studies are now underway to determine the nature of this change in synaptic current and its cellular basis.

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178.3 EMBRYONIC ORIGINS OF THE GIANT FIBER SYSTEM IN CRICKETS. G.A. Jacobs and R.K. Murphey, SUNYA, Albany, N.Y. 12222

We have examined the embryonic origin of a class of mechanosensory interneurons (INs) to learn more about the development of this system and provide a framework within which to study segmentally homologous neurons within and across species. These bilaterally symmetric INs are located in the terminal ganglion, which is composed of fused embryonic ganglia 7-11. The fusion process tends to distort the positions of cell somata and commissures, so we stained INs throughout this embryonic period to insure a correct interpretation of their segmental origins.

In embryos the IN somata are confined to three distinct clusters in each ganglion. Neurons within a cluster cross the midline in the same commissure and project rostrally in either a ventromedial tract (VMT) or a dorsolateral tract (DLT). Somata of neurons in cluster 1 are located in the anterior dosolateral quadrant of their embryonic ganglion, cross the midline in the anterior commissure (AC) and project anterior in the VMT. Cluster 2 somata lie ventromedially, their neurites cross the midline in the AC and project anteriorly in the DLT. Cell bodies of neurons in cluster 3 occupy the posterior dorsolateral quadrant of their ganglion cross dorsally in the posterior commissure and travel rostrally in the DLT. This pattern of clusters is repeated for ganglia 7-10; ganglion 11 however, contains only a single cluster of neurons. These resemble cluster 1 neurons, their neurites form a single commissure and project anterior in the VMT. Ganglion 11 appears reduced to an "anterior half" ganglion as compared to the others.

We can now use this information from embryos to devise a classification scheme for many previously identified neurons. Each neuron is given a segment number followed by a cluster number which reflects its embryonic origin. For example: neurons 7-1, 8-1(MGI),and 9-1(LGI), belong to cluster 1 in their respective ganglia, cross in the AC and have axons in the VMT. Neuron 11-1 was originally described by Levine and Murphey (1980) as a 10th segment neuron, our results show that it comes from the 11th segment. This numbering scheme incorporates without change cricket neurons 9-2,10-2,9-3,10-3 of Mendenhall and Murphey (1974). These studies also allow us to identify segmentally homologous neurons, for example, the positional IN described by Sakaguchi and Murphey (1981) is located in cluster 2 in the 7th ganglion and we have identified its segmental homologues in ganglia 8-10. We are now in a position to study the receptive field properties of these homologous neurons to determine how segmental position affects synaptic connectivity.

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178.4 EMBRYONIC DEVELOPMENT OF THE GRASSHOPPER'S CERCAL SENSORY SYSTEM: ORIGIN OF SENSORY NEURONS AND THE FORMATION OF THE CERCAL NERVE. Marty Shankland and David Bentley. Depts. of Molecular Biology and Zoology, University of California, Berkeley, Ca. 94720.

Antibodies against horseradish peroxidase display high-affinity binding to some unknown antigen(s) found in insect neurons (Jan & Jan, Soc. Neurosci. Abstr. 7:3, 1981). In grasshopper embryos differentiating neurons begin to express this antigen around the stage of growth cone formation, and we have used direct immunofluorescence to follow the development of the peripheral nervous system in the cercus. Antibody staining shows that the first cercal neurons differentiate at 45% of embryonic life. These cells migrate from the epidermis into the lumen, where they form apical dendrites and send afferent axons toward the CNS. Eventually these cells become associated into two scolopidial organs, one located at the tip of the cercus and one at the base. The two goups of axons grow separately along the ventral body wall toward the CNS, and converge onto a pair of more proximal neurons, where they fuse to form a single afferent nerve. These proximal neurons--the Midway Cells--differentiate at 35% of embryogenesis at a point halfway between the cercal bud and the CNS, and are the first peripheral neurons to take part in the construction of the cercal nerve.

Between 50 and 65% the antigen appears in a large number of sensory neurons located in the cercal epidermis. These epidermal neurons send afferent axons into the lumen, where they fasciculate onto the nerve already established by the scolopidial organ at the tip. Anti-peroxidase faintly stains two other cells, the trichogen and tormogen cells, which are associated with each epidermal neuron. These two cells will respectively form the shaft and socket of a hair receptor innervated by the neuronal dendrite. While the sensory neuron undergoes morphological differentiation coincident with the appearance of the antigen, we found that the trichogen and tormogen cells do not form the sensory hair until one molt later in development. During the intervening time the epidermis secretes an embryonic cuticle which is without sensory hairs. The dendrites of the sensory neurons form specialized contacts with this cuticle, and when the cuticle pulls free from the epidermis at 70% the dendrites remain attached and lengthen to span the gap. At 80% the trichogen cell extends a process into the space beneath the old cuticle to form the hair shaft around the dendrite, and at 85% a new, postembryonic cuticle is secreted over both hair and socket. The old cuticle plus that portion of the dendrite not encased within the hair shaft are shed at hatching. Thus, during grasshopper embryogenesis epidermal sensory neurons appear one molt prior to the formation of their sensilla.

178.5 CHOLINE ACETYLTRANSFERASE-DEFICIENT MUTANTS OF THE NEMATODE C. ELEGANS: EFFECTS ON NEUROMUSCULAR JUNCTIONS. J. B. Rand*, O. J. Bashor*, L. F. Cavalier*, and R. L. Russell* (SPON: I. Hanin). Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have obtained 5 independent allelic mutations in the gene cha-1, which we believe to be the structural gene for choline acetyltransferase (ChAT). Four of these alleles (b401, p1152, p1154, and p1156) are relatively severe, and strains containing them share the same spectrum of phenotypes: greatly reduced ChAT activity (0.4-2.0% of wild-type), slow growth, small size, uncoordinated movement, and resistance to cholinesterase inhibitors (carbamates and organophosphates). The fifth allele, p503, is relatively mild; strains homozygous for p503 have 8-10% the normal ChAT level and are behaviorally and developmentally normal. The residual ChAT activity in strains homozygous for p503 has altered K_m's and salt sensitivity; these results together with the strict gene dosage observed with animals heterozygous for any of the alleles support our contention that cha-1 is the structural gene for ChAT (or a subunit of ChAT). Animals homozygous for any of the severe cha-1 alleles have acetylcholine levels decreased about 50%, which, although significant, are not nearly as profound as the decrease in ChAT activity.

Since the nervous system of C. elegans is simple (350 neurons total) and invariant, and since all the synaptic interactions have now been mapped, it is possible to examine known, identifiable putative cholinergic neurons in both wild-type and mutant animals. Therefore, we investigated the effects of genetic reductions in acetylcholine synthesis on the formation of (cholinergic) NMJ's and on neuronal circuitry in general through serial-section electron microscopic reconstruction of the nerve cords of wild-type and cha-1 animals. Series of 200-1000 sections (72.5 nm/section) were analyzed; all the motor neurons in both the ventral and dorsal nerve cords were identified and traced, and all their neuromuscular output was catalogued. The appearance, structure, and circuitry of the motor neurons from the cha-1 strains were normal. However, we observed a reproducible 30-35% decrease in the frequency of cholinergic NMJ's in cha-1 animals (.60-.66 NMJ's/µm in cha-1 vs .94-.99 NMJ's/µm in wild-type). We do not know if this reduced neuromuscular output is sufficient to account for the uncoordinated behavior of these mutants, but we have also observed alterations in the synapse patterns of ventral nerve cord interneurons in cha-1 animals, and the exact circuitry in these mutants is being ascertained.

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178.6 Genetic Studies of Sensory Neuron Projection Patterns in Drosophila melanogaster. S. H. Green. Division of Biology 216-76, Caltech, Pasadena, CA 91125.

The projections of genetically produced ectopic thoracic sensilla into the brain of D. melanogaster were studied by cobalt and HRP filling. These were compared with the normal projections of these sensilla into the ventral ganglion as well as the projections into the brain of the local sensilla that normally occupy the new locations of the ectopic neurons. The ectopic sensilla studied were the tarsal and tibial bristles of antennal legs produced by the genotype Antp Df(3)sbd /Pc ss and dorsal thoracic and wing margin bristles produced by the mutation ey . The mutations cause these sensilla to appear on the head by replacing or being added to sensilla that are normally present.

The normal antenna's projection has also been described by Stocker, R. and Lawrence, P. (Dev. Biol. 82, 224-237, 1981). It consists of a bilateral olfactory lobe (OL) component organized into glomeruli, and a mainly ipsilateral component in the posterior deutocerebrum and SEG which can be further subdivided into 3 major branches. The head macrochaetes all arborize in an identical manner in the subesophageal ganglion (SEG). The routes they take to the SEG, however, vary according to their positions on the head. The arborization is L-shaped with an ipsilateral, longitudinally oriented branch and a medially directed branch that crosses the midline. One branch of the non-OL antennal component precisely matches the head bristle projection.

The antennal leg projection is identical to the antennal for the non-OL component but in the OL the axons arborize randomly, are not organized into glomeruli, have few contralateral branches and have adventitious projections into the protocerebrum and SEG. Some of these latter are consistent from fly to fly. Mechanosensory fibers alone can make up the entire projection.

Thoracic and wing bristles on the head and eye project in the brain and optic lobes. In the brain they often reach the SEG but ramify irregularly. Similarly, their branching in the optic lobes is irregular and unlike that of retinular or intrinsic axons.

I conclude that the ability of ectopic axons to be guided by local pathways or branch in local neuropil is not like that of local neurons and depends on the modality or type of ectopic sensilla that is being studied. In general only the initial projection of the ectopic sensilla is like the local sensilla. The projections of ectopic sensilla into "foreign" neuropil bears no resemblance to their normal projections.

179.1

WITHDRAWN

179.2 CELL BIRTHDAYS IN THE SYMPATHOADRENAL SYSTEM: RELATION TO SURVIVAL AND DIFFERENTIATION IN DISSOCIATED CELL CULTURE.
P. Claude, L.E. Lillien*, I. Parada* and S.K. Presto*.
Wisconsin Regional Primate Research Center and Neuroscience Training Program, University of Wisconsin-Madison 53706.

In order to use neurons and adrenal chromaffin cells in vitro as models for differentiation in vivo, it would be useful to know whether the cells that survive in culture are representative of the donor population or whether they represent a subpopulation with special properties. This is a concern in situations in which a small proportion of the donor cells survive in culture. In this experiment we asked whether there was a selection in vitro on the basis of the developmental age of the cells . We used 3H-thymidine (3HT) labeling to compare the proportion of labeled neurons or chromaffin cells in dissociated cell cultures with the proportion of labeled cells in the donor tissues. Female rats at different stages of pregnancy (from E15 through E20; gestation is 21 days) received a single intraperitoneal injection of 3HT. When litters were born, cultures of dissociated cells were prepared from the superior cervical ganglia (SCG) and the adrenal medullae of some of the pups. After 5 days of growth in vitro in the presence of nerve growth factor, cultures were fixed, air dried, and coated with Kodak NTB-2 emulsion. SCG and adrenal medullary tissues from newborn littermates were also prepared for autoradiography. Chromaffin cells in 1 µm Epon sections of adrenal glands and neurons in sections of the SCG were identified by morphological criteria and the proportion of labeled cells was determined. In the adrenal cultures, chromaffin cells were identified on the basis of catecholamine histofluorescence and the proportion with neurites was noted.

The proportion of labeled neurons in the SCG in vivo was maximal following injections on E15 (23%) and declined progressively to 4% following injections on E20. In culture we observed a similar pattern of decline in the proportion of neurons labeled between E15 (34%) and E20 (0.7%), with a trend toward the preferential survival in culture of older neurons, i.e., those born before E18. The pattern of chromaffin cell labeling was quite different. In the adrenal in vivo 9% of the chromaffin cells were labeled following injections on E15 and the proportion of labeled cells rose to 19% following injections on E20. Preliminary analysis of chromaffin cell cultures suggests that, as in the case of cultured sympathetic neurons, there was some preferential survival of older chromaffin cells. However, there did not appear to be a large bias, based on developmental age, in the selection of neurons or chromaffin cells that survived in culture.

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179.3 EXPRESSION OF NEURONAL TRAITS IN CULTURED NEURAL CREST.
J. Girdlestone and J.A. Weston (SPON: G. Ciment). Dept. of Biology, Univ. of Oregon, Eugene, OR, 97403.

Neural crest cells give rise to a number of derivatives including the neurons and glia of the PNS. To characterise the pattern of differentiation of these neurons we have examined the appearance of several neuronal markers in cultured neural crest populations. One such marker, the monoclonal Ab E/C8, which binds to neurons of the PNS and CNS, reveals the presence of a small sub-population of antigen-positive cells in primary neural crest cultures. These labelled cells are generally found in spherical aggregates, but antigen-positive cells with fine processes are also seen amongst the monolayer of non-neuronal cells, mostly melanocytes, which constitutes most of the culture. Catecholamine-fluorescent cells are also detected in crest cultures and a double-labelling procedure is being developed to determine if these constitute a sub-population of the cells containing the E/C8 antigen. With the availability of such specific markers we hope to assess the relative roles of environmental and intrinsic factors in the process of differentiation.
This work was supported by NIH grant DE-04316, and a grant from the Dysautonomia Foundation Inc.

179.4 INTRACELLULAR MIGRATION OF MONOCYTES THROUGH THE CHOROID PLEXUS AS POTENTIAL EPIPLEXUS (KOLMER) CELLS IN THE MONKEY.
R. G. Clark. Dept. Anatomy and Cell Biology, Univ. Miami, Sch. of Med., Miami, FL 33101.

It is well known that a unique population of macrophages adheres to the surface of the choroidal epithelium. First called Kolmer cells, they now are usually referred to as epiplexus cells. Phagocytic in nature they have elongated processes which interdigitate with the microvillus processes and cilia of the choroid plexus epithelial cells. It has been suggested that these cells are hematogenous in origin and pass from the connective tissue core to cross between epithelial cells to reach the ventricular surface. The present study provides the first evidence that the macrophages pass through the epithelium but that the migration occurs by an intracellular rather than an extracellular route.

Six normal rhesus monkeys (2-4 kg) were studied. The animals were sacrificed with an overdose of sodium pentobarbital and perfused through the heart with 6 liters of a solution containing 1% paraformaldehyde and 1% glutaraldehyde in 0.075 M cacodylate buffer. Small portions of choroid plexus from all ventricles were removed and processed for electron microscopic evaluation.

The most significant finding was the presence of monocytic, macrophage-like cells which appeared to be passing through the choroid plexus. At times they were observed in the intercellular space between adjacent epithelial cells but the zonula occludens was always intact and appeared to block any further migration. Generally, the macrophages were contained within the choroidal cells causing the latter to bulge prominently into the ventricular lumen. The epithelial cells were so engorged by the monocytes that their nuclei were indented and cytoplasmic organelles greatly compressed. The macrophages did not appear to be phagocytizing the epithelial cells. Structurally the macrophages looked similar to the epiplexus cells but lacked the long cytoplasmic processes.

180.1 EARLY DESCENDING NEURONS OF THE XENOPUS TAIL SPINAL CORD. R.H. Nordlander. Depts. Anatomy and Oral Biology, Case Western Reserve University, Cleveland, OH 44106.

Fiber tract development in the amphibian spinal cord begins with the ingrowth of axons into small spaces between adjacent neuroepithelial cells (Nordlander and Singer, Exp. Neurol., 75:221, 1982). Each fascicle at first consists of one or a few axons to which others are added. The rostrocaudal developmental gradient, which allows this sequence to be observed within individual specimens over a range of stages, and the simplicity of the tail spinal cord makes it especially suitable for the study of pathfinding in the vertebrate central nervous system.

The purpose of this study was to identify the earliest descending axons of the tail spinal cord and to describe the cell classes to which they belong. Horseradish peroxidase (HRP) was applied to the spinal cord of Xenopus (stages 30 to 39) at a level previously shown to receive the first descending axons. Specimens were incubated for 10 min. to 48 hr. before fixation, reacted with the method of Hanker, et al. (Histochem. J., 9:789, 1977), embedded in plastic and sectioned in transverse and horizontal planes. HRP traveled retrogradely along axons of the two earliest axonal bundles, one dorsolateral (DLF) and one ventrolateral (VLF), to fill cells located rostral to the application site. Among these cells several types can be clearly distinguished on the basis of position and morphology.

Rohon-Beard cells are the most commonly labeled cells at the earliest stages examined. These cells, which are part of the primary sensory system, occupy a dorsal position and send ascending and descending axons in the DLF in addition to a peripheral axon.

Primary motor cells are smaller neurons appearing in the ventrolateral mantle layer. Each of these cells sends a short process rostrally and a longer axon caudally in the VLF. The descending process exits the cord via a ventral root at a more caudal level. Dendrites spread radially into the lateral marginal layer.

The most prominant and earliest interneurons to appear are the dorsolateral cells which are located directly lateral to the Rohon-Beard cells. Each of these cells sends a stout process circumferentially along the lateral border of the cord, later to be displaced medially as the marginal layer expands. Dendrites extend laterally from the process before it divides to send ascending and descending processes into the VLF. At successive stages the proportions of labeled interneurons increases and at least three types are observed at stage 39.

- The morphologic development of these cells was followed by comparison of specimens incubated for progressively longer periods.

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180.2 INNERVATION OF CHICK WINGS WITH A SPLIT LOWER ARM: AN EVIDENCE FOR ACTIVE NERVE-MUSCLE RECOGNITION. M. Neset, C. Muniak* and C. Edwards. Neurobiology Research Center, SUNY Albany, Albany, N.Y. 12222.

Experiments were designed to acquire more information about the development of pattern formation. Innervation was studied in 12 day embryonic chick wings, having two lower arms in "parallel", both originating from the elbow. The grafts were performed according to Saunders and Gasseling (1968, In: Fleishmajer and Billingham, R.E. (Eds.) Epithelial-Mesenchymal Interactions).

The two flexor carpi ulnaris muscles (f.c.u.) of the grafted limb were always innervated, and the cell bodies of the neurons were in the specific area for f.c.u.-neurons in the cord, as determined by the retrograde HRP-technique. There are at least three possible explanations for this result. The innervation could be due to an increase in the number of neurons innervating the duplicated lower arm, i.e. a decrease in cell death. However, the numbers of motor neurons on the experimental and control side are similar, and this seems to exclude a role for cell death. Alternatively, branching of the neurons prior to entering the muscles may be present, with both muscles innervated by the same nerve fibers, keeping the number of motor neurons constant. However, the results of direct stimulation of the nerve trunks at the spinal root and close to the muscle showed no evidence of branching. Therefore, it is likely that the f.c.u.-muscles are innervated by separate groups of neurons, and that each fiber innervates more muscle fibers than normally.

The present findings taken together with earlier results of proximo-distal grafted limbs (Muniak & Neset, 1981, Soc. Neurosci. 7:464; Neset & Edwards, 1981, Soc. Neurosci. 7:465), show that a specific group of neurons innervates the f.c.u.-muscles whether these are in "parallel" or in "series", and that the total number of motor neurons is not affected by these types of grafts.

Supported by the Muscular Dystrophy Association.

180.3 MONOCLONAL ANTIBODIES TO CEREBELLAR AFFERENT INPUTS, Richard Hawkes*, Evelyn Niday, Gerda Huber* and Andrew Matus. Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

There are two afferent pathways to the mammalian cerebellum, the climbing fibers which terminate on the Purkinje cell dendrites and the mossy fibers which synapse with granule cell dendrites. We have produced a library of monoclonal antibodies (mab) which recognize diverse components of the rat cerebellum. Among these are two classes which are apparently specific for one or other afferent system. On SDS-gel immunoblots the first class, exemplified by mab 12D5, recognizes a single polypeptide band of apparent molecular weight 270,000. Immunocytochemistry reveals that staining of the cerebellum is confined to the granular layer, primarily to the synaptic glomeruli. Similar studies of neonatal tissue suggest that the staining locus is the mossy fiber system. Consistent with this hypothesis, we have identified antigen-positive cell bodies and processes in the pons, a major source of mossy fibers. The second group, exemplified by mab 6A1, recognizes several distinct polypeptide bands on gel blots. The immunocytochemical staining with 6A1 is confined to the molecular layer where it is distributed as patches along the margins of the Purkinje cell dendrites, consistent with the size and distribution of climbing fiber synapses.

180.4 HISTOGENESIS OF THE RAT SUBSTANTIA NIGRA: ISTHMIC ORIGIN OF ITS NEURONS. R. Marchand. Centre de recherche en Neurobiologie, Hôpital de l'Enfant-Jésus, 1401, 18e Rue, Québec, Qué. G1J 1Z4.

This study provides new data on the time of origin, the site of generation and the route of migration of the young neurons of the substantia nigra of the rat during embryogenesis. The neurons of the substantia nigra are generated on day 12, 13, 14 or 15 of gestation. They settle following a light spatiotemporal rostrocaudal gradient from day 12 to day 15. The neurons of the substantia nigra are generated at two different points of the basal plate at the level of the fovea isthmi (meso-isthmic junction) and migrate in radial pattern as two definite streams toward the ventral mesencephalon. Then the two streams of cells move rostralwards along the surface towards their final site. The median stream of cells originate in the medial basal plate along the unpaired floor plate of the isthmus rhombencephali. The median stream of cells clearly contributes to the formation of the substantia nigra, pars compacta, the area of Tsai and to the nucleus linearis caudalis. The ventrolateral stream of cells originates at the same level although more laterally along the basal plate. It contributes cells to the lateral and ventral areas of the susbtantia nigra.

The main findings of this work are the disclosure that the neurons of the substantia nigra are generated in the region of the isthmus rhombencephali and that its cells do not migrate between existing cells of the mesencephalic tegmentum as claimed previously but first migrate ventralwards in a radial pattern and then rostrally towards their definite site. All the cell groups of the basal mesencephalon and the midline structures of the caudal mesencephalon are apparently derived from the region of the isthmus rhombencephali.

180.5 CHANGES IN CENTRAL CANAL AREA IN YOUNG RATS FOLLOWING EXPOSURE TO X-RAYS. S. A. Gilmore, T. J. Sims and J. E. Leiting*. Depts. of Anatomy and Pathology, Univ. Arkansas Med. Sciences, Little Rock, AR 72205.

A population of cells having nuclei resembling those in the lateral walls of the central canal form a well-defined cluster situated just ventral to the central canal area. This ventrally situated cell cluster is extremely sensitive to ionizing radiation (Soc. Neurosci. Abst., 7:467, 1981). By 6 hours post-irradiation (P-I) many nuclei in this cluster are pyknotic, and by 24 hours this cluster essentially disappears. Further light and electron microscopic studies have been in progress to analyze the changes occurring in this area following exposure to x-rays.

A beam of soft x-rays (4000R) was directed to a 5mm length of lumbosacral spinal cord in 3-day-old rats. For light microscopy the rats were perfused-fixed at intervals from 6 hours to 10 days P-I. The spinal cords were embedded in JB4, and a variety of stains was used. Normal 3-day-old rats and rats killed at 18 and 24 hours P-I were used for ultrastructural studies. These animals were perfused with a modified Karnovsky's fixative, and both thick and thin sections were prepared in the usual fashion.

Ultrastructurally the cells in the ventral cluster in the normal 3-day-old rat resembled glioblasts, probably astrocytes. The nuclei were generally ovoid, with moderate amounts of condensed chromatin around the nuclear envelope. The cytoplasm was of medium density and contained microfilaments. Extensions of this cytoplasm surrounded small bundles of axons. The perikaryal membranes had no synapses or junctional complexes.

The ventral cluster, which had virtually disappeared by 24 hours P-I, was not reconstituted by 10 days P-I. During this interval, the cellular organization in the irradiated animals changed from a pseudostratified to a simple arrangement in the lateral walls of the canal, suggesting that a cell loss had occurred. Cell counts showed that the walls of the canal in the irradiated rats contained approximately 40% fewer cells than in the controls. Pyknosis was not a prominent feature of the lateral walls light microscopically. However, degenerative changes were observed ultrastructurally in the laterally situated ependymal cells. These data indicate that the cells in these areas (ventral cluster vs. lateral wall) are differentially sensitive to effects of ionizing radiation.

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180.6 IS NEURAL MATURATION SIMILAR IN PRECOCIAL AND ALTRICIAL MURIDS? P. C. Brunjes. Dept. of Psychology, University of Virginia, Charlottesville, Va. 22901.

Offspring of Acomys cahirinus (the "spiny mouse") are born after a 38 day gestation period with open and functional ears and eyes and sophisticated locomotor capabilities. Would an animal with such divergent development exhibit ontogenetic patterns similar to those found in more commonly studied altricial murids such as the laboratory mouse and rat? The long gestation period of Acomys suggests that rates of maturation in altricial and precocial pups are the same with birth occurring at some arbitrary timepoint. To test this notion, we examined olfactory bulb growth in 0, 10, 20, and 60 day postpartum Acomys pups. The bulb was chosen because its clear lamination and pattern of late maturation facilitated examination. The following measures were employed: 1) changes in main olfactory bulb laminar volume (glomerular, G; external plexiform, E; mitral, M; and combined internal plexiform, inner granule and ventricular, I), 2) accessory olfactory bulb volume and 3) mean glomerular size. Tissue from four animals per age was examined and all measurements made with an Apple II based computer system. Significant age-related increases were found in all measures except for the mitral layer. Most increases were found between Day 0 and Day 10 (percentage increase was 73 for G, 156 for E, 50 for M, 72 for I, 81 for the accessory olfactory bulb and 80 for glomerular size) indicating that large amounts of postnatal maturation do occur in the precocial brain. Hinds and Hinds (J. Comp. Neur., 169:20, 1976) report that mice of the conceptual age examined here (postnatal days 18-28) also exhibit increases in bulbar volume, with most occurring in the external plexiform layer. However, mice evidenced only a 40% increase, compared to the 156% change found in Acomys. Thus, compared to their altricial cousins, Acomys exhibit considerably more neural growth at late gestational ages. Our results suggest that brain development is not similar in altricial and precocial murids; Acomys undergoes more neural development than predicted by data from altricial pups, and that neural maturation is delayed in Acomys in relation to the development of other systems. Perhaps neural growth and differentiation are withheld until after parturition to allow newborns to adapt to a complex world.

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180.7 A SIGNIFICANT FRACTION OF THE ADULT NUMBER OF MATURE CEREBELLAR PURKINJE CELLS FIRST APPEARS BETWEEN POSTNATAL DAYS 16 AND 30 IN THE MOUSE. T.J. Diglio* and K. Herrup, Dept. of Human Genetics, Yale Medical School, New Haven, CT 06510

The achievement of the adult number of most large neuronal cell types is generally assumed to occur early in development. One obvious corollary to this view is that it should be possible to quantitatively account for all members of a given cell type at all stages following their ventricular genesis (and subsequent period of cell death, if any). We report here that rigorous quantitative analysis of cerebellar Purkinje cells does not bear out this prediction. Specifically, we find a 40% increase in Purkinje cell number occurs in the mouse between postnatal days 16 (P16) and P30.

Two to four C57BL/6J mice were analyzed at each of eight postnatal ages ranging from P10 to P180. All animals were perfused with half strength Karnovsky's fixative and processed identically. Serial wax sections of cerebellar halves from each animal were assembled, stained and, in 12 to 15 sections from each series, all Purkinje cells with a clearly visible nucleus were counted (5000 to 7500 cells per animal). Correcting our raw counts by the method of Hendry, with slight modifications, we find the number of Purkinje cells remains constant from P10 to P16 (51,000 cells per half cerebellum) then increases over the ensuing two weeks to reach adult levels (74,000 cells) by P30. Several additional observations lend perspective to these findings. First, Caddy and Biscoe (Proc. Roy. Soc. Lond. 287, 167) made nearly identical observations in C3H mice but dismissed their finding as artifact. Second, cells of the facial nucleus in the same animals used for the Purkinje cell counts described above do not have a period of cell number increase during postnatal life. Lastly, the number of medium-to-large neurons (MLNs) in the staggerer mutant also show no postnatal increase in cell number. Since the staggerer mutation is known to block Purkinje cell development at early stages, this finding suggests that the 40% increase in the wild-type is part of the normal sequence of developmental events.

Although the source of the late Purkinje cells remains unknown, preliminary analysis of animals injected embryonically with ^{3}H-thymidine and examined autoradiographically at several postnatal ages demonstrated that these cells do not arise by late development of immature cells present in the Purkinje cell layer at P10. Whatever their source, the occurence of this late addition of a large fraction of major neuronal cell type suggests a new chapter must be added to the story of CNS develoment.

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180.8 TRANSIENT VISUALIZATION OF β-ENDORPHIN-CONTAINING NEURONS IN THE RAT BRAINSTEM DURING PERINATAL DEVELOPMENT. G. Baetge, W.J. Shoemaker, A. Bayon, R. Azad, and F.E. Bloom. The Salk Institute, La Jolla, CA 92037.

We have previously described the development of the opioid peptides, β-endorphin and enkephalin, in diencephalic and telencephalic structures of the rat. At embryonic day 16 (ED-16) β-endorphin is detectable in both brain and pituitary. Using permeation chromatography followed by radioimmunoassay (RIA), only a small amount of high molecular weight material (β-LPH or 31K protein) is present in the whole brain at this early stage of development (Bayon et al., Soc.Neurosci.Abstr.5:523, 1979). We now describe further details of this system using both immunocytochemical staining and high performance gel permeation chromatography on the brain stem. Using anti-serum specific for β-endorphin and the indirect 2-Step immunoperoxidase technique, we see stained perikarya and processes in the dorsal medulla oblongata coincident with nucleus commissuralis. The staining could be blocked by pretreatment with authentic β-endorphin and was observed from late fetal stages to post-natal days 17 or 19. The β-endorphin-positive cells are dispersed throughout an arc from the midline dorsal to the central canal which extends to the dorso-lateral edge of the vagus nucleus. Their distribution is similar to the dopamine-β-hydroxylase-positive staining cells of the A_2 catecholamine group, but the two groups have different morphological shape and appear to be separate. The perikarya of the β-endorphin-positive neurons are ovoid in shape with processes extending from either one or both poles. The longitudinal axis of the cells and the stained processes orient along the arc-shaped zone of immunoreactive cells. We are currently utilizing HPLC gel-permeation chromatography to resolve the different molecular weight components in this and other regions of embryonic, newborn and post-natal animals. Preliminary results indicate that the β-endorphin has the size of authentic β-endorphin with no high M.W. β-endorphin I.R. It is not yet clear whether the loss of β-endorphin positive perikarya in the brain-stem group after postnatal day 19 results from "programmed cell death", a switch to production of another neurotransmitter or simply loss of peptide stores to a level below detection. (Supported by NIAAA 03504 and NIDA 01785.)

180.9 GOLGI ANALYSIS OF NEURON DIFFERENTIATION IN THE PRESUMPTIVE DORSAL HORN OF THE E12 MOUSE EMBRYO. L. E. Wentworth. Dept. of Anat., Med. Ctr., Sch. of Med., San Francisco, CA 94143

During embryonic day 12 (E12, where EO = day vaginal plug observed) the presumptive dorsal funiculus reaches approximately 2/3 across the top of the dorsal cord and the ventricular layer comprises less than ½ the width of the dorsal cord. In addition to commissural cells and ipsilateral association cells, which began differentiating on E9 and have been described previously (Wentworth and Yee, Anat. Rec., 193:717-718, 1979), a variety of neuron types are differentiating in the alar plate region at this time.

Transversly oriented fusiform cells are seen which border the dorsal extent of the presumptive dorsal horn. Unlike ventral horn motoneurons, commissural and ipsilateral association neurons which go through a unipolar stage followed by a bipolar stage with the first dendrite growing from the pole opposite the axon, most of these cells go from a unipolar stage to a multipolar stage by branching first at the axonal pole and later at the opposite pole. These cells probably represent the marginal cells of lamina I in the adult.

Elongated pyriform cells oriented dorsoventrally are located in the mid intermediate region of the alar plate at this time. The axon is directed dorsally in some of these cells and ventrally and/or toward the lateral funiculus in others. A few cells are seen in the mid intermediate region which resemble early pyramidal cells. They are also oriented dorsoventrally and have an apical dendrite , basal dendrites and a descending axon. Some cells in the dorsal intermediate region have a tangential orientation with an axon directed dorsally and dendrites directed laterally toward the dorsolateral funiculus.

Multipolar cells are seen in the dorsolateral intermediate region which have extensive dendritic networks branching into the dorsolateral funiculus. These cells may be similar to some cells described in lamina IV of the adult (Brown, Organization in the Spinal Cord, Springer-Verlag, 1981).

The axon of many cells of the dorsal cord ramifies, sometimes extensively. Occasionally a cell is seen to have an axon which bifurcates and enters both the presumptive dorsal funiculus and the dorsolateral funiculus. Only a limited number of very early stages of neuron differentiation in the alar plate have been seen to date with the rapid Golgi method. We are now serially reconstructing thin sections in order to obtain a more complete picture of the differentiation of these cell types.

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180.10 SEXUALLY DIMORPHIC DENDRITIC ORGANIZATION IN THE PREOPTIC AREA OF THE NEONATAL RAT. P. E. Meyers* and J. H. Gordon (SPON: C. M. Combs). Departments of Anatomy and Pharmacology, The Chicago Medical School, N. Chicago, IL 60064.

The dorsomedial portion of the medial preoptic area has been described as being sexually dimorphic. The current study examines normal development of sexually dimorphic dendritic organization in the rat pup within this area.

Six millimeter square blocks of tissue were removed from pups at 2, 4, and 6 days of age. Blocks were prepared by the Golgi-Cox method, embedded in celloidin, and cut at 70u. Camera lucida drawings of well-impregnated neurons were obtained from the dorsomedial medial preoptic area. Neurons were analyzed with a grid composed of concentric rings divided into equal octants. Rings were in radial increments of 10.5 um. Octants were numbered from 1 to 8 in a clockwise direction starting from 12 o'clock. At this time, data from the 6 day rats (2 male and 2 female) is the most complete. Data was taken as ring crossings per octant of each sex. For this preliminary analysis, crossings per octant are expressed as percentages of total crossings.

Sex	Total crossings	Octants (crossings/total crossings)							
		1	2	3	4	5	6	7	8
Female (20 cells)	378	31	12	8	7	20	10	2	10
Male	297	25	6	8	18	15	10	6	13

Females showed a strong preference for octant 2, differing from males by 52%. Females showed a minor preference for octants 1 and 5. Dendrites from females tend to orient from dorsolateral to ventromedial. Males showed strong preferences for octants 4 and 7, differing from females by 61% and 66.6% respectively. Males showed a minor preference for octant 8. Dendrites of males tend to orient from mediodorsal to ventrolateral. The younger animals showed similar trends.

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180.11 THE DEVELOPMENT OF MEISSNER'S CORPUSCLES IN PRIMATE DIGITAL SKIN. W. E. Renehan* and B. L. Munger* (SPON: W. Graham). Dept. of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA 17033.

The development of Meissner's corpuscles has been studied in digital skin of fetal, newborn and adult rhesus monkeys (Macaca mulatta). Specimens were taken from 14 cm (measured crown-rump), 15 cm, 16 cm, 17 cm, 18 cm, and 19 cm fetal monkeys, covering the period of 3-6 months of gestation, and from newborn and adult animals. Serial paraffin sections were stained with ammoniacal silver, and tissue for electron microscopy was prepared using conventional methods.

Early in the third month of gestation, developing Meissner corpuscles were represented by a close association of one to five thinly myelinated and unmyelinated axons with the dermal capillary loop, high in the dermal papilla. As development proceeded, the vascular elements assumed a position lower in the papilla. Each axon branched into a number of smaller diameter fibers, oriented vertically with respect to the dermal papilla. In 15 and 16 cm fetuses, the axons pierced the basal lamina of the epidermis at the top of the papilla, and penetrated into the stratum basal and stratum spinosum. The portions of these axons in the apex of the dermal papilla were associated with a cluster of presumptive lamellar cells. These lamellar cells were indistinguishable from the Schwann cells associated with the afferent axons. During the fifth and sixth months of gestation the basal lamina of the epidermis was discontinuous in the region of the apex of the dermal papilla, allowing cell contact between lamellar and epidermal cells. In the neonatal period the basal lamina was again continuous.

In the perinatal fetus, the axons supplying the maturing corpuscles were no longer present in the epidermis. Instead, the thin unmyelinated branches of the parent axons changed their orientation, and assumed a position parallel to the skin surface. At the same time, the cytoplasmic processes of the lamellar cells occupied the spaces between the horizontal terminal portions of the afferent axons. The corpuscle was surrounded by a perineurial capsule continuous with the perineurial sheath of the nerve bundle. The capsule, however, was discontinuous at the apex of the dermal papilla. Axon terminations were enlarged, filled with numerous vesicles, mitochondria, neurofilaments, and membranous elements.

During the first month of life, an increase in the number of lamellae caused the Meissner corpuscle to achieve maturity.

(Supported in part by USPHS HD-11216.)

181.1 REDUCTION OF NATURALLY-OCCURRING CELL DEATH IN THE THORACO-LUMBAR AND SACRAL PREGANGLIONIC CELL COLUMN OF THE CHICK EMBRYO FOLLOWING BLOCKADE OF GANGLIONIC TRANSMISSION. J.L. Maderdrut* and R.W. Oppenheim (SPON: W.E. Hall). Neuroembryology Lab., Dorothea Dix Hospital, Raleigh, N.C. 27611.

The thoraco-lumbar (sympathetic) and sacral (parasympathetic) preganglionic cell column (column of Terni, CT) is formed by the secondary migration of motoneurons from the common motor column (CMC) in the ventrolateral spinal cord to a mediodorsal position between Stage 25-26 (day 4.5-5) and Stage 34 (day 8). Nerve fibers from perikarya in the cervical and thoraco-lumbar CMC were detected in the primary sympathetic chain by Stage 25. There was a significant loss of motoneurons in the thoraco-lumbar and sacral CT between Stages 34 and 36 (day 10). Pyknotic (degenerating) cells were seen in both the thoraco-lumbar and sacral CT during the period of naturally-occurring motoneuron loss. A massive degeneration of neurons occurred in the cervical CMC between Stages 24 and 26.

Either presynaptic (hemicholinium-3, HC-3) or postsynaptic (pempidine) blockade of ganglionic transmission spanning the period of naturally-occurring cell death reduced both the loss of motoneurons and the number of pyknotic cells in the thoraco-lumbar and sacral CT. Ganglionic blockade had no apparent effect on the volume of the thoraco-lumbar sympathetic ganglia at Stage 36. Blockade of ganglionic transmission between days 10 and 15 (Stage 41) decreased the volume of the thoraco-lumbar sympathetic ganglia but had no effect on the number of visceromotor neurons in the corresponding CT. Numberous small pyknotic profiles (presumably, oligodendrocytes) were scattered through both the gray and white matter of the spinal cord following treatment with HC-3. Blockade of ganglionic transmission between days 3 (Stage 18) and 8 had no effect on either the massive cell death in the cervical CMC or the secondary migration of thoraco-lumbar and sacral visceromotor neurons. The three drug regimens had no effect on the nuclear diameter of either thoraco-lumbar or sacral visceromotor neurons.

Both competitive (curare) and depolarizing (decamethonium) neuromuscular blockade between days 5-6 and 10 reduced naturally-occurring cell death in the thoracic medial (somatic) motor column but had no effect on cell death in the thoracic CT (cf. R.W. Oppenheim and J.L. Maderdrut, Proc. Soc. Neurosci., 1981, 7, 291). Postsynaptic blockade of ganglionic transmission had no effect on cell death in either the thoracic medial motor column or the lumbar lateral (somatic) motor column.

181.2 SURVIVAL OF LATERAL MOTOR COLUMN NEURONS IN EXPLANT CULTURES: TARGET AND SUBSTRATUM INFLUENCES. E. D. Pollack. Inst. Study Develop. Disabil., University of Illinois, Chicago, IL 60608.

Growth and survival of nerve fibers from developing tadpole spinal cord cultures have been demonstrated to be dependent on the presence of mesenchymal limb target tissue and the nature of the substratum (Pollack, Neurosci. Lett.,'80; Pollack et al., J. Comp. Neurol.,'81; Muhlach and Pollack, Dev. Brain Res.,'82). Increased survival of nerve fibers from cord explants has assumed the increased survival of lateral motor column (LMC) neurons. This is now confirmed for the LMC cells of spinal cord explants from young frog tadpoles cultured in the presence of limb mesenchyme and on the alternative substrata of collagen or polylysine. Stage V (Taylor-Kollros stages) tadpole lumbosacral spinal cord explants were cultured for 21 days on either re-constituted rat tail collagen (COL) or on poly-DL-lysine (PLYS) in the presence or absence of stage V mesenchymal hindlimb and then serially sectioned and stained. Control cord explants grown alone on PLYS exhibited an average LMC cell count 10 times that of explants on collagen. On both substrata, the LMC cell count was increased significantly when limb explants were present. However, the increases were more dramatic on collagen since very few LMC cells survive in cord cultures on collagen that lack target tissue. These findings are consonant with previous results on nerve fiber outgrowth.

The preliminary results suggest that both a target-based influence and the nature of the substratum may act interdependently to regulate neuronal survival in vitro. On collagen the limb target seems to mimic the survival effects from PLYS alone, while the addition of limb to cord cultures on PLYS enhances even further the survival of the LMC neurons. Increased numbers of cells are attributable to survival rather than proliferation since mitotic index and DNA differences are of insignificant magnitude. The present morphological observations correlate well with biochemical indices for tadpole spinal cord explants under similar conditions (Muhlach and Pollack, Neurosci. Abstr.,'82). Aside from neuronal counts, it appeared that the distribution of necrotic cells within the explants was a consistent feature. Only infrequently were such cells located within the LMC when limb had been present or in the ventricular zone, but were otherwise evenly distributed throughout the intermediate zone. These results offer further evidence in support of the hypothesis that the mesenchymal limb target serves as a source of a motor neuron survival (and growth) factor, while the attachment substratum acts in concert with it to favor the factor-promoted activities of the neuron.

(Supported by NIH grant NS 13814.)

181.3 TARGET DEPENDENCY OF CEREBELLAR GRANULE CELLS ON PURKINJE CELLS. QUANTITATIVE ANALYSIS OF LURCHER CHIMERIC MICE. R. WETTS & K. HERRUP, (SPON: E.L. Giller, Jr.). Dept. of Human Genetics, Yale Univ. Sch. Med., New Haven CT 06510.

Lurcher heterozygous mice (+/Lc) suffer a loss of 100% of the cerebellar Purkinje cells (PCs) and 90% of the granule cells (GCs) between the second and fifth postnatal weeks. To determine which neurons are directly or indirectly affected by the Lc gene, aggregation chimeras were constructed. The nervous systems of chimeric mice develop by the intermixing of mutant and wild type cells. In lurcher chimeras, the numbers of PCs and GCs are intermediate between +/Lc and wild-type mice. Previous work has shown that the Lc gene acts intrinsically on the PCs to cause their death, whereas the degeneration of the GCs is a secondary event.

Since the PCs (the only known direct site of Lc gene action) begin to degenerate during the period of synaptogenesis, it seems likely that the GCs die because they are unable to form synapses with their major post-synaptic partner. To investigate this theory further, we determined the numbers of GCs and PCs in 2 +/Lc, 4 wild-type and 3 lurcher chimeric mice. Since each of the 4 wild-type mice was a different inbred strain, the number of PCs was different in each. The numbers of GCs also differed such that there was a positive correlation with the numbers of PCs. When GC number is plotted as a function of PC number, the 4 points of the wild-type mice fall on a line that intercepts the y axis at zero GCs. This suggests that the number of GCs in a given animal is regulated by the number of PCs, possibly because the survival of the GCs is dependent on the formation of synapses with the PCs. Further, there appears to be little plasticity in this system; each PC can only support a specific number of GCs (about 180). The GC vs PC graph for the 3 chimeras also forms a straight line. This line has the same slope as the wild-type line, which lends some support to the above model. However, the y-intercept of this chimera line is 6.5 million. We assume that during the first week of synaptogenesis, the lurcher chimeras have a normal complement of PCs (just as +/Lc mice do), and this may partially explain the extra 6.5 million GCs. The reason this excess is greater than 1.5 million (the number of GCs in +/Lc mice) is unknown. It may indicate some plasticity in the chimeric environment affecting the interaction of GCs with PCs. Thus, the cerebellar GCs are similar to other neurons for which survival is dependent on the post-synaptic target. Supported by NIH grants HD07149, HD 12213 and NS 18381 and by the March of Dimes Birth Defects Fund.

181.4 THE SHORT AND LONG-TERM EFFECTS OF MOSSY FIBER - DEAFFERENTATION OF THE LATERAL CEREBELLAR CORTEX OF THE RAT. W.A. Anderson* and B.A. Flumerfelt (SPON: J.A. Kiernan). Department of Anatomy, University of Western Ontario, London, Canada.

Electrolytic lesions made within the basilar pontine gray resulted in the degeneration of nearly the entire population of mossy fiber rosettes within the ansiform lobule. These degenerating varicosities represented two distinct populations with respect to their time course of degeneration. The majority of the rosettes, simple and complex dispersed types, underwent a slow course of electron-dense degeneration. As a result, most glomeruli contained debris from degenerating mossy fiber varicosities during the first 57 days. A second, small population of mossy fiber rosettes of the simple clustered variety, however, underwent a rapid course of electron-dense degeneration by the 5th day. Since electrolytic lesions of the basilar pontine gray also included the nucleus reticularis tegmenti pontis, this center could represent the source of the simple clustered variety of mossy fiber rosette. All glomeruli were denervated by 80 days following pontine lesions.

Animals deafferentated of their mossy fiber input as adults were allowed to survive for a period of 57-120 days and subsequently examined following Golgi impregnation or processing for electron microscopy. The primary response to mossy fiber - deafferentation was the transneuronal degeneration of the granule cell system. Morphological evidence is provided which suggests that the mossy fiber varicosities may play an important role in the fragmentation and the removal of the granule cell digitiform dendrite. Image analysis of Golgi impregnated Purkinje cells has indicated significant losses in both smooth branch and spiny branchlet numbers following the loss of mossy fibers. Ultrastructural evidence for transneuronal degeneration which supports the quantitative findings is provided. The transneuronal degeneration of both the molecular layer interneurons and the Purkinje cells following mossy fiber - deafferentation, however, is secondary to the transneuronal loss of the granule cell system. Although a loss of dendritic spines occurred along the terminal branchlets following mossy fiber - deafferentation, many of the existing spines underwent marked changes in form. The present findings suggest that denervated Purkinje cell dendritic spines elongate to acquire a new synaptic input. (Supported by the MRC of Canada)

181.5 AGE-RELATED ULTRASTRUCTURAL CHANGES IN SYNAPTIC DEGENERATION IN OLFACTORY CORTEX: A BASIS FOR PLASTICITY. R.A.E. Bakay* and L.E. Westrum. Depts. of Neurological Surgery and Biological Structure, Univ. of Washington, Seattle, WA 98195.

The age dependent capacity of the olfactory cortex to reorganize and the form that this reorganization takes following deafferentation may be related to the comparative degenerative response of the synaptic structures at the various stages of synaptic maturation. The different patterns of these degenerative responses are being studied electron microscopically in rats receiving ipsilateral olfactory bulb ablation at birth, 3, 6, 9, and 13 days of age. Survivals include acute (12-36 hrs), subacute (3-7 days), intermediate (10-14 days), and chronic (up to 30 days) times. Acutely degenerating axon terminals in layer I are predominantly watery and flocculent in character in the 1-6 day old operants and are removed with minimal glial response subacutely from their postsynaptic contacts. No evidence of degeneration is observed at chronic survival times. This immature pattern of degeneration and possible competitive reinnervation is similar to that observed in newborn rats with the same lesion (Westrum, Anat. Embryol., 160, 1980). Electron-dense degeneration of synaptic terminals is observed first in the 6 day old operants subacutely, then becomes the predominant form in the 9 day old operants, and is the exclusive pattern in the 13 day old operants. The dense degenerating material increases in amount after the 6 day old operants with the 13 day old operants exhibiting a mature adult pattern of persisting degeneration (Westrum, Z. Zellforsch., 98, 1969). There is a marked glial phagocytic response in the 9 day old operants that increases with age. Growth cones and other processes invaginate dense terminals and possibly reestablish new contacts. Postsynaptic degeneration rarely seen in the 6 day old operants is common in the 9 day old operants, and is extensive in the 13 day old operants. This parallels neuronal death presumably from transynaptic degeneration in layer II which is again rare in the 6 day old operants and increases to an adult pattern in the 13 day old operants. The results show dramatic differences in the form of terminal degeneration between 3 and 13 operants coupled with increased glial activity, greater numbers of persisting dense degenerated terminals, and onset of neuronal death. All elements of the adult patterns of degeneration are present in the 6 day old operants to a minor degree and in the 9 day old operants this becomes the predominant pattern. It might then be predicted that these would represent critical ages at which major changes occur in the potential plasticity of the olfactory cortex. (Supported by N.I.H. Grants No. NS 09678, NS 17111-01S1 and DE 04942. LEW is also an affiliate of the CDMRC.)

181.6 EARLY RESPONSES OF CAT RETINA TO VISUAL CORTEX ABLATION AT BIRTH. Helen E. Pearson, Bert Payne and Timothy J. Cunningham. Depts. of Anatomy and Physiology/Biochemistry. The Medical College of Pennsylvania, Philadelphia, PA 19129.

Some neurons in the central nervous system of young mammals are especially vulnerable to target removal after lesions while others which survive often have a remarkable capacity to reorganize their projections. We have investigated these phenomena in the retina of infant cats following visual cortex lesions; such lesions remove most of the lateral geniculate nucleus, a major target for optic axons. As a result, there is both ganglion cell death, more marked in the periphery than in central retina, (Pearson et al. 1981) and reorganization of retinofugal pathways (Labar, et al. 1981). Normal retinae and retinae from kittens with all cortical visual areas ablated bilaterally at birth (day 1) were compared in the light and electron microscope. The animals were perfused on postnatal days 2-5. Whole mounts were prepared and four blocks of retina were taken along the vertical meridian from periphery to center. Every 50th semithin section was collected through the blocks from the left eye and stained with toluidine blue. Blocks from the right eye were trimmed for thin sectioning. We examined peripheral retina for evidence of both naturally occurring and lesion-induced cell death. We examined central retina, where ganglion cells are more likely to survive the lesion, for fine structural changes. In peripheral retina of normal kittens, late stages of cellular degeneration are found in both the ganglion cell layer and the inner nuclear layer. The appearance of these profiles in normals suggests that naturally occurring neuron death is a feature of postnatal development of the cat's retina. In central retina of normal kittens, fine structural observations indicate that ganglion cells accumulate cisternae of rough endoplasmic reticulum (RER) and polysomes from postnatal day 2 to day 5. By day 3, Nissl-like bodies are apparent in the cytoplasm of some cells. In operated animals, degenerating cells appear also in the ganglion cell and inner nuclear layers. Preliminary counts from peripheral retina of 2 and 3 day-old kittens show an increased surface areal density of degenerating profiles in both layers of the operated animals. In the central retina of operated animals, ganglion cells contain some cisternae of RER but often have large areas of cytoplasm occupied by monosomes. The cytoplasmic changes are seen clearly 72 hrs after the lesion and are similar to the reaction shown by neurons during regenerative sprouting. The results suggest that after visual cortex ablation retinal ganglion cells respond rapidly. Some die, but others reorganize their intracytoplasmic organelles possibly to support modified growth of the subcortical visual pathways.

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181.7 RETINAL GROWTH AND NEURONAL DEATH IN THE DEVELOPMENT OF ADULT DIFFERENTIAL GANGLION CELL DENSITY. D.R. Sengelaub and B.L. Finlay. Department of Psychology, Cornell University, Ithaca, N.Y. 14853

At maturity the hamster retina contains approximately 80,000 ganglion cells. These ganglion cells are distributed unevenly across the retinal surface with a central elevation in density of 2:1 over peripheral areas. To understand how this central specialization develops, we have studied the distribution of cells in the ganglion cell layer through the first 10 postnatal days with light microscopy.

On the day of birth, cell counts in horizontal sections through the retina found the linear density of cells in the ganglion cell layer to be essentially uniform across the retinal surface ($\bar{x}$ central= 78.1 cells per 100u; $\bar{x}$ peripheral= 82.9 cells per 100u; central-peripheral ratio= .94). By postnatal day 10 this distribution approaches the adult pattern ($\bar{x}$ central= 34.4 cells per 100u; $\bar{x}$ peripheral= 24.6 cells per 100u; central-peripheral ratio= 1.4). By postnatal day 10 there is a 226% decrease in the central cell density and a 336% decrease peripherally. Since glia are added to the retinal ganglion layer during this early postnatal period, these postnatal day 10 cell densities underestimate the actual decrement in neuron density. During the first 10 postnatal days the retinal cross-sectional length increases by 260% and this growth is responsible for part of the decrease in density. However, considering the glial addition, growth alone is unlikely to account for the density changes centrally and cannot account for those seen in the periphery.

The hamster retinal ganglion cell layer shows a substantial amount of cell death during this same period and cell death rates in the periphery of the retina are 25% higher than those found centrally. We suggest that this differential cell death can account for the additional decrease in the peripheral cell density.

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181.8 INCREASED EARLY INNERVATION OF THE SUPERIOR COLLICULUS DOES NOT RESULT IN INCREASED CELL SURVIVAL. Kenneth C. Wikler* and Barbara L. Finlay. Department of Psychology, Cornell University, Ithaca, N.Y. 14853

Following partial unilateral lesions of the superior colliculus in neonatal hamsters, neuroanatomical and electrophysiological investigations have demonstrated that the entire retina projects in an orderly fashion to the remaining tectal area in the adult. This tectal fragment could thus be hyperinnervated by the retina in early development. Since our prior studies have shown that substantial cell death occurs in the superior colliculus in this period, this preparation provides a model to test if increased afferent innervation can decrease the amount of early cell loss in a target structure.

Lesions of the caudal half of the right tectum were made on the day of birth in neonatal hamsters. Reductions of the surface area of the residual superficial gray layer of the superior colliculus ranged from 48% to 79% of normal surface area. The numbers of degenerating cells, recognized by their pyknotic appearance in light microscopy, were expressed as a fraction of the number of normal-appearing cells. These ratios were then compared between the normal and early-ablation sides. Assessments of ratios were made for postnatal days 4-8, the period of maximal early cell death in the hamster superior colliculus. No differences in either the time course or the amount of early cell loss were found (mean ratio for normal side =4.22 degenerating cells per 1000 normal cells; mean ratio for the early ablation side= 3.93 degenerating cells per 1000 normal cells; F=0.21,ns).

These results suggest that increased afferent innervation does not sustain more neurons in a target population, and that in normal development, targets are saturated with afferents that must compete for terminal space.

Supported by NSF BNS 79 14941

181.9 INDUCED CHANGES IN THE LATERALITY OF THE CENTRIFUGAL PROJECTION TO THE AVIAN RETINA. Dennis D.M. O'Leary and W. Maxwell Cowan. The Salk Institute, and The Clayton Foundation for Research-California Division, La Jolla, CA 92037.

In normal post-hatched chicks the nucleus of origin of centrifugal fibers to the retina, the isthmo-optic nucleus (ION), contains about 10,000 neurons, all of which send their axons to the contralateral eye. In addition there are approximately 1,500 cells that lie outside the boundaries of the ION, which can be retrogradely labeled by injections of various marker substances into the eye. Of these "ectopic ION neurons", all but a small percentage (≈ 2%) project to the contralateral eye. Early in the development of the nucleus (around the 12th day of incubation - E12) the ION and ectopic neurons also project predominantly to the contralateral eye; the exception being that in addition to the small percentage of the ectopic neurons which project to the ipsilateral eye about 1% of the ION cells also project ipsilaterally. These ipsilaterally projecting ION neurons can no longer be labeled after E17. However, if one optic vesicle or optic cup is removed between stages 10 and 17 (of the Hamburger and Hamilton series) and wheat germ agglutinin-conjugated HRP (WGA-HRP) is injected into the surviving eye on E12 in many of the cases essentially all of the cells in the ION of both sides are labeled. In chicks from which an optic cup was removed on or after stage 13 and WGA-HRP is injected into the other eye on day E18, the ION on the contralateral side appears heavily labeled, but that on the opposite side has virtually disappeared. Counts of the number of labeled cells in the contralateral ION indicate that in these cases often only 50% of the normal number of ION neurons survive. This suggests that during the period when axons from the ipsilateral ION were present in the surviving retina (between sometime late on the 9th day, when they first reach the retina, and some time after day 12) they compete with the axons from the contralateral ION, either for available synaptic sites or more probably for a trophic agent provided in limited quantities by the retina, and although their parent cells subsequently degenerate, during the period of the competition they jeopardize the survival of about half of the population of ION neurons on the contralateral side. Interestingly, if the eye removal is done at stage 12 or earlier a substantial number of ION and ectopic ION neurons (up to 3,400) persist on the ipsilateral side and can be retrogradely labeled by an intraocular injection of WGA-HRP at E18. On the contralateral side in these cases there are again only about 50% of the normal number of ION and ectopic ION neurons. It is noteworthy that in all of these cases in which a significant percentage of the ipsilateral ION neurons survive anterograde labeling has revealed the presence of a substantial ipsilateral retino-tectal projection.

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181.10 THE EFFECTS OF PROTEIN DEPRIVATION ON THE NUCLEUS LOCUS COERULEUS: A MORPHOMETRIC GOLGI STUDY IN RATS OF THREE AGE GROUPS. S. Diaz-Cintra*, L.Cintra*, T.Kemper* and P.J.Morgane. (Spon: W.McFarland). Worcester Foundation for Expt. Biology, Shrewsbury, MA 01545.

In our continuing morphometric Golgi studies of the monoamine nuclei of the brainstem we have examined the effects of an 8% protein diet, instituted prenatally, on the three cell types we previously characterized in the nucleus locus coeruleus (Brain Res., in press). In control rats fed a 25% protein diet all cell types (ovoid, fusiform and multipolar) in the locus coeruleus showed significantly decreased dendritic spine densities on both primary and secondary dendrites between 30 and 90 days of age. All three cell types then showed a significant increase in spines between 90 and 220 days. In protein malnourished animals we previously found that the nucleus raphe dorsalis shows a significant dendritic spine incrementation between 30 and 90 and between 90 and 220 days (Brain Res. 221: 243-255, 1981) thus indicating that this nucleus responds to malnutrition by continuing to add dendritic spines across the 3 age groups studied. The three cell types in the locus coeruleus in animals fed a low protein (8%) diet show a significant decrease in primary and secondary dendritic spines between 30 and 90 days followed by a highly significant increase in spines between 90 and 220 days with the highest increase in dendritic spines during this period occurring in the multipolar and ovoid cells. Thus, locus coeruleus cells respond to malnutrition with an initial slight decrease in dendritic spines between 30 and 90 days followed by a highly significant increase between 90 and 220 days of age. It is apparent from these studies that the nucleus raphe dorsalis and locus coeruleus each respond to malnutrition insult with an increased synaptic input with the course of spine development in locus coeruleus in the period 90-220 days of age being in the same general direction as that of the raphe dorsalis cells, i.e., toward a higher dendritic spine density. Protein malnutrition thus appears to markedly affect the monoamine nuclei of the brainstem. Further, the response of these cells to malnutrition is in the direction of keeping and/or adding new dendritic spines in coping with such a stress. (Supported by NFH Grant HD-06364, NICHD)

181.11 SEGMENTAL SELECTIVITY OF SYNAPSE ELIMINATION IN RAT SOLEUS MUSCLE. Wesley J. Thompson. Dept. of Zoology, Univ. of Texas, Austin, TX 78712

I have examined the segmental selectivity present in the elimination of synapses from rat soleus muscle by comparing the extent of synapse loss by soleus motor neurons in each of the two spinal nerves (L4 and L5) supplying the muscle. For this purpose, motor unit sizes were measured by stimulating single soleus motor axons teased from the L4 and L5 ventral roots and comparing the size of the twitch or tetanic contraction evoked to that obtained by direct stimulation of the muscle. Intracellular recordings of epp's in muscle fibers while stimulating single L4 or L5 soleus motor neurons showed this tension measurement procedure gave a reasonable estimate of the percentage of the muscle innervated by a motor axon. I found that L4 and L5 motor units were, on average, about the same size at 6 days post-natal and that units in both nerves underwent an average four-fold reduction in size during subsequent synapse elimination. Thus, individual L4 and L5 motor neurons lose approximately the same numbers of synapses. However, the whole L4 spinal nerve was found to lose synaptic contact with more muscle fibers than the whole L5 nerve, as shown by both tension recordings and intracellular recordings of epp's. This apparent disproportionate loss by the L4 spinal nerve was shown to be explained by the smaller number of soleus motor neurons present in L4 compared with L5. These results were obtained from three different Wistar rat stocks. Therefore, in contrast to reports by others (Miyata and Yoshioka, J. Physiol. 309: 631; O'Brien, J. Physiol. 317: 89P), I find that synapse elimination in rat soleus shows no marked segmental disproportion. Rather, as initially shown by Brown et al. (J. Physiol. 261: 387), synapse elimination results in the generation of motor units of approximately the same size by favoring the loss of synapses by the larger motor units, irrespective of spinal origin of motor neurons.

181.12 THE REGULATION OF SYNAPTIC POSITION, SIZE, AND STRENGTH IN ANURAN SKELETAL MUSCLE. Bruce Nudell* and Alan D. Grinnell. (SPON: A. Cangiano). Jerry Lewis Neuromuscular Res. Center, UCLA, Los Angeles, CA 90024.

In an attempt to discern the rules governing synaptic size and the competitive interactions among endplates on multiply innervated muscle fibers, we have examined the physiology, morphology, and position of endplates on identified fibers in the Xenopus laevis pectoralis muscle. This work has revealed that:

1) The percentage of fibers with one endplate declines in large muscles; and within the same muscle, singly-innervated fibers are smaller than dually-innervated fibers.
2) Single junctions tend to be stronger than junctions on dually-innervated fibers.
3) Single junctions are typically located near the middle of their fibers, while the endplates on dually-innervated fibers are located toward either end, and are usually separated by at least 20% of the total fiber length.
4) Junctions on the same dually-innervated fiber tend to be more similar in length than do junctions on different fibers of the same input resistance. There is no corresponding tendency for greater similarity in physiological strength of paired junctions, which frequently show large differences in EPP amplitude.
5) The total terminal length on dually-innervated fibers of equivalent input resistance is inversely correlated with the mean release/length and total release of both junctions. There is no apparent correlation between the distance separating endplates and their strength or length.

The data provide support for a model of synaptic regulation similar to that proposed by Jansen and his colleagues (Jansen, J. K.S., Thompson, W., & Kuffler, D.P., Prog. Brain Res. 48: 3-18, 1978) in which nerve terminals are attracted, grow and are maintained in proportion to the amount of a substance supplied by muscle fibers. Our findings suggest that such a substance is produced or distributed uniformly throughout each fiber in amounts proportional to the fiber size and inversely proportional to the total transmitter output of all junctions innervating the fiber or the level of muscle fiber activity they evoke. A form of competitive interaction between the terminals which helps to determine synaptic spacing may involve local depletion of this substance.

181.13 SYNAPSE REPRESSION IN CULTURE. M.C. Fishman, A.E. Schaffner and P.G. Nelson. Lab. Dev. Neurobiol., N.I.H., Bethesda, MD 20205.

Selective reduction in polyneuronal innervation is characteristic of the developing neuromuscular junction. It seemed possible that the use of cell culture might provide an opportunity to evaluate this phenomenon at the molecular level. We have studied the cholinergic synapses between ciliary ganglion neurons and myotubes in culture, and the effects of spinal cord neurons upon these synapses.

The ciliary ganglion of 8-day chick embryos was explanted onto chick myotubes plated 2 weeks previously. By 3 days after plating the ciliary ganglion had extended a halo of neurites. Synaptic connections were assessed by intracellular recording from myotubes while stimulating the ganglion extracellularly through a 5-10μm tip micropipette filled with 0.9% NaCl. Recordings were obtained from all muscle cells within one field diameter (~500μm) of the ganglion (4-10 muscle cells per plate). There was no clear correlation between the degree of obvious morphological neurite-myotube contact and presence of synaptic connections. Under control conditions between 80 and 100% of myotubes evidenced synaptic input from the ciliary ganglion. This degree of innervation remained stable over four days (days 3 to 6 after plating).

In other plates we added dissociated spinal cord cells from 6-day embryos 1 week prior to adding the ciliary ganglion. We enhanced this spinal cord population for motoneurons by dissociating only the ventral half of the cord. The concomitant presence of these ventral spinal cord cells only slightly reduced the number of myotubes manifesting connections to the ciliary ganglion at the time of first evaluation. Thus, 82% of myotubes exhibited epsp's 3 days after plating of the ciliary ganglion in the presence of spinal cord cells, while 96% of myotubes tested in control plates were connected. Over the ensuing days there was a progressive decrement in the ciliary ganglion-myotube synapses, with 64% of myotubes connected at day 4, 62% at day 5, and 17% at day 6. Experiments in which similar numbers of dissociated cells were added from the dorsal spinal cord or from the dorsal root ganglion showed that these cell populations did not cause a reduction in ciliary ganglion-myotube synapses over the same time period. The medium alone, previously conditioned by spinal cord cells and myotubes, also did not cause a reduction in synapses, suggesting that the synapse repression was not due to removal of critical synapse stabilization factors from the medium by the spinal cord cells.

Thus, synapse repression can take place in culture, even without the intricate connections and full heterogeneity of cell populations found *in vivo*.

181.14 DENDRITIC SPINES IN LOCUS COERULEUS AND NUCLEUS RAPHE DORSALIS DEVELOP OUT-OF-PHASE IN RATS OF THREE AGE GROUPS. Peter J. Morgane, Tom Kemper*, Leon Cintra* and Sophia Diaz-Cintra* (Spon: A. Dren) Worcester Found. for Expt. Biol., Shrewsbury, MA 01545.

We have carried out morphometric Golgi studies on the three cell types (ovoid, multipolar and fusiform) which we identified in the nucleus raphe dorsalis (Brain Res. 207: 1-16, 1981) and nucleus locus coeruleus (Brain Res., in press). Between 30 to 90 and 90 to 220 days of age dentritic spines on both primary and secondary dendrites of all three cell types in each nucleus showed exact out-of-phase development between the two nuclei. However, all three cell types within each nucleus were precisely in phase with each other with respect to their dendritic spines. All three cell types in the nucleus raphe dorsalis between 30 and 90 days showed a significant increase in dendritic spines on primary and secondary dendrites (16% increase on fusiform cells; 24% increase on multipolar cells; 15% increase on ovoid cells). The locus coeruleus during this period showed a significant decrease in spines on primary and secondary dendrites (21% decrease on fusiform cells; 16% decrease on multipolar cells; 39% decrease on ovoid cells). Between 90 and 220 days of age all three cell types in the locus coeruleus showed a significant increase in dendritic spines (50% on fusiform cells; 18% on multipolar cells; 67% on ovoid cells). In the nucleus raphe dorsalis there was a significant decrease in dendritic spines on two of the three cell types (9% decrease on fusiform cells; 19% decrease on multipolar cells), the exception being a non-significant decrease in primary dendritic spines in the ovoid cells (decrease of 9%). This out-of-phase development of dendritic spines between locus coeruleus and dorsal raphe may have important functional implications in a wide variety of behaviors via their widespread projection systems and are thought to be reciprocally inhibitory.
(Supported by NIH Grant HD-06364 and NSF Grant BNS 79-22507)

182.1 THE DORSAL MOTOR NUCLEUS AND NUCLEUS AMBIGUUS IN THE CAT AND MONKEY: LIGHT AND ELECTRON MICROSCOPIC STUDIES FOLLOWING HRP INJECTIONS INTO THE VAGUS NERVE. J.H. McLean and D.A. Hopkins. Dept. of Anatomy, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

A recent study in the cat has shown that the dorsal motor nucleus of the vagus nerve (DMV) may contain three neuronal types with differing projections (McLean and Hopkins, J. Comp. Neurol., 1982). The nucleus ambiguus (NA) also has a variety of cell types but its fine structure has not been studied with respect to efferent projections. In the present study the fine structure and projections of the DMV in the Cynomolgus monkey have been compared to those in the cat and the NA has been studied in both the cat and monkey after injections of horseradish peroxidase (HRP) into the cervical vagus nerve. The brain stems were processed for light and electron microscopy using standard procedures and tetramethylbenzidine and diaminobenzidine were used as chromogens for HRP histochemistry.

After HRP injections in the vagus nerve, retrogradely labeled cells were present ipsilaterally in the DMV, NA, intermediate zones and occasionally in the medial solitary nucleus. In the monkey DMV, medium-sized neurons measuring 26 x 18 µm with abundant cytoplasm and an oval nucleus with few indentations were labeled. Small neurons measuring 12 x 9 µm with scanty cytoplasm and an invaginated nucleus were not labeled and accounted for 12-18% of the DMV neurons. In this respect, the monkey DMV resembles that in the cat. At the electron microscopic level, axosomatic synapses were observed on both types of neurons. Most terminals were 1-2 µm in diameter. Approximately 70% of the terminals that synapsed on medium-sized neurons contained round vesicles and made symmetric (RS) or asymmetric (RA) contact with the plasmalemma while 30% contained pleomorphic vesicles and made symmetric (PS) contact. On small neurons, over 90% of the synapses were of the RA or RS type.

In both species, the NA contained labeled neurons dispersed among unlabeled neurons. Many labeled neurons were large (45 x 35 µm) but the majority averaged 30 x 20 µm. These neurons were round or spindle-shaped with an oval or indented nucleus and an abundant cytoplasm. Smaller labeled neurons (24 x 18 µm) with an invaginated nucleus were more common in the rostral NA. Axosomatic synapses were observed on the plasmalemma of almost all labeled neuronal profiles. Generally, the terminals were 1-2 µm in diameter. Approximately 50% of the terminals formed RA or RS type synapses while the remainder were PS type.

These results provide a basis for further ultrastructural studies on the connectivity and structural organization of the cells of orgin of the vagus nerve in the cat and monkey. Supported by the MRC of Canada.

182.2 STAPEDIUS AND TENSOR TYMPANI MOTONEURONS IN THE CAT. M. Shaw and R. Baker. Dept. Physiol. and Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016

The numbers and locations of motoneurons to the middle ear muscles, stapedius and tensor tympani, were determined using retrograde transport of horseradish peroxidase (HRP). Stapedius motoneurons lay outside the traditionally recognized facial nucleus in several distinct locations: 1) in the interface between the facial nucleus and the superior olive: 2) in a thin scattered lamina of somewhat smaller cells spread dorsal to the facial nucleus; and 3) in a cluster located ventro-medial to the rostral third of the facial nucleus. Smaller numbers of cells also lay dorsal to the superior olive or scattered in the reticular formation, medial to the descending loop of the facial nerve. All projections were ipsilateral.

Tensor tympani motoneurons also lay outside the traditionally recognized trigeminal motor nucleus, in an area 200µm ventral to it. At the medial edge they formed a closely packed group, but at the lateral edge the cells trailed ventrally, medial to the trigeminal motor root. They extended beneath the full rostro-caudal length of the trigeminal motor nucleus. Filled cells were also found in the inferior salivatory nucleus, probably from spread to the lesser superficial petrosal nerve which clings to the muscle epimysium in the cat. Some HRP filled cells were also found in the trochlear, oculomotor and mesencephalic nuclei. The labeling of these cells could not be explained by spread of HRP through the Eustachian tube and into the orbit. First, the trochlear projection was entirely ipsilateral. Second, neurons to tensor veli palatini and the pterygoids were not labeled, as would have been expected. Labeled trochlear neurons were seen in every case in which the whole muscle was injected and may represent a small direct innervation of the tensor tympani or surrounding structure. HRP filled axons could be seen crossing the pontine midline, but their source could not be ascertained.

The mean number of motoneurons was 696 for tensor tympani and 668 for stapedius, yielding innervation ratios of 1:5.7 and 1:2.6, respectively. These values place stapedius and tensor tympani among the most finely innervated muscles known.

Thus, the motor pool for a cranial muscle need not be organized as a distinct subnucleus of the major motor nucleus of its branchial arch. These findings also raise intriguing questions about the development of specific innervation either to or from motor pools as widely scattered as stapedius and tensor tympani. Supported by NIH #EY0504, Fight-For-Sight, NYC #F-346 and USPHS #NS13742.

182.3 EXCITATORY ACTION OF CYCLOBENZAPRINE IN LOCUS COERULEUS (LC) AND SUB-COERULEUS (SC). N. Penington* and R. J. Reiffenstein. Dept. of Pharmacology, Univ. of Alberta, Edmonton, AB, Canada T6G 2H7

Opposite mechanisms of action for the tricyclic, central skeletal muscle relaxant cyclobenzaprine (CBZ) have been advanced. Depression of spontaneous and evoked firing of LC neurons has been reported by Barnes and co-workers (1980, 1981) who proposed that blockade of norepinephrine (NE) uptake enhanced recurrent inhibition of LC. Commissiong et al. (1981) reported CBZ increased LC firing and turnover of NE in ventral spinal cord, and loss of the relaxant effect of CBZ after LC destruction. They proposed that CBZ blocked α_2-adrenoceptors on LC neurons. We have attempted to investigate these differences by systemic and local drug administration in urethane-anesthetised (1.2-1.5 G/KG i.p.) 150-250 G male Sprague-Dawley rats. Only data from histologically identified recording sites (after staining with Pontamine Sky Blue ejected from the electrode) are reported; most were in SC and ventral LC. CBZ (0.25-0.5 mG/KG, i.v.) greatly increased LC/SC neuron firing in 35 of 38 animals. After CBZ, noxious stimulation still induced vigorous LC/SC firing, but normal rebound decreases in firing (post-stimulus suppression, PSS) were seldom seen. Low doses (0.25 mG/KG, i.v.) of yohimbine (YOH) produced the same effect in all 11 trials. Also, clonidine (CLON)(10-75 µG/KG, i.v.) reduced spontaneous firing and the firing in response to CBZ or noxious stimulation. Since all drugs used alter blood pressure (BP), and BP is known to affect LC firing (Persson and Svensson, 1981), this relationship was studied in 12 animals. We observed LC/SC firing and BP were inversely related. Spontaneous changes in LC/SC firing were mirrored by BP changes. CLON-induced inhibition of, and PSS of, LC/SC firing were paralleled by increases in BP, while the vasodepresssor effects of CBZ and YOH coincided with increased firing. Indirect drug effects via BP were avoided by applying NE, CLON, CBZ and YOH iontophoretically. Both CBZ and YOH (5-30 nA) potentiated the response to noxious stimulation, increasing the duration of the LC/SC firing (blockade of PSS), and sometimes also increasing the maximal rate. Induction of firing in the absence of stimulation was generally not seen. Very high doses of YOH (>80 nA) and CBZ (>150 nA) suppressed the maximal firing rate and the (extracellular) spike height. NE and CLON inhibited LC firing, consistant with previous observations. We conclude that a direct effect of i.v. CBZ on LC cannot be shown, but the iontophoretic results are consistant with an α_2-antagonist action of CBZ on LC neurons. The absence of direct stimulation in response to local CBZ suggests that the effects are due to potentiation of physiologically evoked LC stimulation by suppression of recurrent inhibition. Supported by U. of A. MR & GR Funds (R.J.R.). N.P. holds an AHFMR studentship.

182.4 AT LEAST 25 DISTINCT BRAINSTEM NUCLEI PROJECT TO THE FOREBRAIN ALONG THE MEDIAL FOREBRAIN BUNDLE (MFB). Robert P. Vertes. Dept. Physiol., Wayne State Univ. Sch. Med., Detroit, MI 48201

In previous reports we found that the discharge of a large percentage of neurons in the medial pontine reticular formation (PRF) was highly correlated with hippocampal theta and that electrical stimulation in this same PRF region was very effective in eliciting a theta rhythm in the hippocampus (J. Neurophysiol. 42:214, 1979 and 46:1140, 1981). It was also shown that a synchronizing system could be traced from PRF through the midbrain in the ventrolateral tegmentum to the diencephalon where it appeared to join the MFB. In the present study HRP injections were made at several levels of the MFB to evaluate the contribution of PRF fibers to the MFB. Small WGA-HRP injections (.25-.50 ug of conjugate) were made in 30 rats and brains were reacted using the TMB procedure of de Olmos et al. (J. Comp. Neurol. 181:23, 1978). Unexpectedly, few HRP reactive cells were found in the PRF following MFB injections. We did observe, however, that a substantial number of PRF cells were labelled when caudal MFB injections encroached upon the supramammillary nucleus (SUM). Further, we have found that SUM is very heavily labelled following HRP injections in both the MFB and the medial septum. These results suggest that the SUM may be an important link between the PRF and the septum in the generation of hippocampal theta.

Although few cells within the medial RF were labelled following HRP injections within the MFB, at least 25 other nuclei in the brainstem were found to contribute fibers to the MFB. These nuclei were the following (from caudal to rostral): A1 (directly above the lateral reticular nucleus); A2 (mainly within the nucleus of the solitary tract); a group of cells on the dorsolateral border of the MLF at the level of the rostral pole of the inferior olive; nucleus gigantocellularis, pars ventralis; raphe magnus; nucleus incertus (on the midline within the central gray at the level of locus coeruleus - LC); dorsal lateral tegmental nucleus of Castaldi (DLT) (between incertus and LC within the central gray - CG); locus coeruleus; nucleus subcoeruleus; raphe pontis; dorsal and ventral parabrachial nucleus; Kolliker-Fuse nucleus; A7 (lateral pontine tegmentum, medial to lateral lemniscus); median raphe; dorsal raphe; rostral and caudal linearis nuclei; retrorubral nucleus (RR) (midbrain tegmentum posterior to red nucleus); ventral tegmental area (VTA); medial part of substantia nigra (SN) pars compacta; peripeduncular nucleus (dorsolateral to SN, pars lateralis); nucleus of Darkschewitsch (ND); a distinct cell group in the midbrain CG dorsal and lateral to ND; and supramammillary nucleus. The median and dorsal raphe, the linear nuclei, VTA, SUM, RR and DLT were heavily labelled. These results suggest that the influence of the brainstem on the forebrain is substantial.

182.5 CYTOARCHITECTURE OF THE PRIMATE PERIAQUEDUCTAL GRAY, NUCLEUS MEDIALIS. L.K. Laemle, UMDNJ- New Jersey Medical School, Newark, N.J. 07103.

The discovery by Reynolds (1969) of the role of the midbrain periaqueductal gray matter in stimulus produced analgesia has renewed interest in the cytoarchitecture and fiber connections of this region. At the present time, data concerning the morphological substrate in this complex, multifunctional area is contradictory. Controversey persists concerning (1) whether data support subdivision of the PAG into discrete nuclei or regions (2) identification of cell populations and their distributions. Regardless of the answers to the above questions, Nissl and Golgi studies in the cat, rat, and monkey, and studies of fiber connections (Mantyh, 1981, 1982), suggest that the cytoarchitecture and organization of the PAG is similar among species. Much of the nociceptive activity appears to be centered in the region identified by Hamilton (1973) as the nucleus medialis (PAGM).

The present report is based upon Nissl, Golgi, and counterstained Golgi preparations of the PAGM in the squirrel monkey, macaque, baboon, and human. Parameters evaluated include cell density and perikaryal size, shape, and orientation. In Golgi preparations additional neuronal characteristics such as axonal origin and trajectory, and the number of primary dendrites, their branching patterns, and spine populations were also studied.

In Nissl preparations, the PAGM is clearly delineated from the remainder of the PAG by the low packing density of its cells. Both Nissl and Golgi preparations indicate that morphologically the primate PAGM is comprised of a heterogeneous population of neurons as judged either by size, or by perikaryal shape and orientation. Three classes of cells are distinguishable based upon perikaryal diameter: small (less than 15uD), medium sized (15u-25uD), and large (greater than 25uD). Using perikaryal shape and orientation as the criteria, neurons can be classified as fusiform (vertical and horizontal), triangular (pyramidal and inverted pyramidal), or stellate. All cell populations described by Laemle (1979) in the human PAGL are present in the PAGM, however, there appears to be a greater proportion of triangular perikarya in the PAGM. Large triangular perikarya (greater than 30uD) which are demonstrated here have not been reported previously. Dendritic arborizations of PAGM neurons are extensive, often spanning the entire PAGM and continuing into the PAGL, or extending through the subependymal zone where they occasionally appear to make contact with the aqueduct. (Supported by GRS funds from UMDNJ-New Jersey Medical School).

182.6 CREST SYNAPSE STRUCTURE AND LOCATION WITHIN THE INTERPEDUNCULAR NUCLEUS OF THE NEAR-TERM RHESUS MONKEY FETUS. N. J. Lenn and V. Wong. Depts. of Neurol. and Pediat., and Clinical Neuroscience Research Center, Univ. of VA, Charlottesville 22908; and Carnegie Labs. of Embryol., Univ. of CA, Davis 95616.

In a previous study, it was found that the neurons of rhesus fetus interpeduncular nucleus (IPN) were generated between E36 and E42 (term = E165). IPN was recognizible on E45. Dendritic branching increased between E81 and P8, suggesting that synaptogenesis would be a feature of this interval. Particular interest in the paired habenular afferent synapses, the crest synapses, is based on their location in particular subnuclei and their consisting of one afferent from each habenula in the rat. In the rhesus, crest synapses have been noted as early as E148, and may yet be found earlier. In the present study, the detailed distribution of crest synapses was mapped at two levels of the nucleus at E162. By this age crest synapses are numerous and of mature fine structure. The more rostral level studied was in the center of IPN, and the more caudal level near its caudal extent. Perfusion fixation followed immediately after delivery by hysterotomy. One % paraformaldehyde-1.25% glutaraldehyde was delivered via the heart. A second solution with twice these concentrations followed. The location of each crest synapse was plotted on photographs on the sections.

Crest synapses were indistinguishable from those of rat. They consisted of two processes containing 40-60 nm synaptic vesicles. These formed asymmetrical contacts, occasionally en passant on opposite sides of markedly narrowed dendritic processes. At the more caudal level, they were concentrated in two intermediolaterally placed zones which curved medially to merge with one another ventrocaudally. These zones were recognizible with rather sharp borders except medially, due to darkly staining fibers. Fibers leaving these areas crossed the central portion of IPN as narrow bundles which separated pallisades of neuronal perikarya. At the more rostral level, there were fewer crest synapses present, but they were similarly located in two intermediolateral zones.

The striking similarities of these findings to those of rat IPN suggest that other features such as neurotransmitter specificity, left-right pairing of afferents at crest synapses, developmental mechanisms which normally produce this pattern, and possibly alterations in synaptogenesis after early unilateral lesions of the habenula, are likely to withstand extrapolation from rat to monkey. Supported by grants HD NS 08658 and RR000169 to the California Regional Primate Center.

182.7 NUCLEAR ORGANIZATION OF THE RAT'S LATERAL THALAMUS. T. Takahashi* (SPON: F. Scalia). Dept. of Anatomy and Cell Biology, S.U.N.Y. Downstate Med. Ctr., Brooklyn, NY 11203.

The lateral thalamus relays visual information from the superficial laminae of the superior colliculus (sc) and the nuclei of the pretectum to the cortical territories adjacent to the striate area. The nuclear organization of this area of the rat has never been precisely determined despite the fact that the rat is often used in behavioral and physiological studies of vision. Analysis of cytoarchitecture and hodology undertaken presently indicates that the lateral thalamus of the rat consists of eight nuclei.

The cytoarchitecture of the lateral thalamus was studied using Nissl-stained, coronal sections of normal brains. To study the innervation of the lateral thalamus, a small injection of ^{3}H-leucine (10-160 nl, 25-50 μCi/μl) was placed in Area 4,17,18, or 18a (of Krieg, J. Comp. Neur., 84:221,1946) or in the sc of female hooded rats (n=34). The brains of these rats were then processed according to the protocol for autoradiography of Cowan et al. (Brain Res., 37:21, 1972). The sizes and positions of the injections were such that virtually all of the areal dimension of Area 17, Area 18 and the superficial layers of the sc was involved.

A study of the cytoarchitecture revealed seven cellular fields which were termed nucleus suprageniculatus (sg), nucleus lateralis posterior pars caudomedialis (lpcm), nucleus lateralis posterior pars lateralis (lpl), nucleus lateralis posterior pars rostromedialis (lprm), intramedullary area, (ima), nucleus lateralis dorsale pars ventrolateralis (ldvl) and nucleus lateralis dorsale pars dorsomedialis (lddm). Analysis of connectivity showed that lpl is further divisible into rostral (lplr) and caudal (lplc) sectors. Therefore, there are eight nuclei in total. Nucleus sg, the most caudal element of the lateral thalamus, is composed of medium to large, fusiform and multipolar neurons. It contains the entire terminal field of the superficial layers of the ipsilateral sc. Nucleus lpcm, found rostrolateral to sg, is loosely packed with large multipolar cells and contains the entire terminal field of the superficial layers of the sc of both sides. Nucleus lpl, a long cellular territory found lateral to lpcm, extends from the caudal pole of the dorsal lateral geniculate (lgd) to the caudal pole of ldvl and contains round cells which are smaller and more densely packed than those of lpcm. Its caudal portion (lplc) contains the entire terminal field of the ipsilateral sc while its rostral portion (lplr) contains that of Area 17. Area 18 projects to lplr and Area 18a projects to both lplr and lplc. The intramedullary area, which occupies the fibrous zone lateral to lpl and medial to lgd, contains round and fusiform neurons and is innervated by Area 18a. Nucleus lprm, situated medial to lpl, is characterized by round neurons which are frequently found in clusters. It contains the entire terminal field of Area 17 and it receives inputs from Area 18 and 18a as well. Nucleus ldvl is evenly packed with moderately large, polygonal cells and contains the entire terminal field of Area 17 and 18. It also receives inputs from Area 18a. Finally, lddm, tightly packed with small round cells, and lying medial to ldvl, receives inputs from Area 4.

182.8 RESPONSE PROPERTIES OF AN INTERNEURON MODEL. D. J. Surmeier* and R. J. Weinberg (SPON: A. L. Towe). Dept. of Physiology and Biophysics, University of Washington, Seattle, Wash., 98195.

As part of a study of the response properties of cells in cat cuneate nucleus, we have developed a neuronal model to assess the possible role of various intrinsic properties of the neuron and of its afferent input in generating spike trains. Most previous neuronal models have assumed that synaptic events preceding the last spike can be ignored in the computation of membrane potential. Thus, immediately following a spike, the membrane potential is restored to its original resting level, and all ongoing synaptic events are eliminated ("membrane resetting"). This "resetting" assumption is not warranted in cells electrically dominated by their dendrites, unless the dendrites support a regenerative action potential. In our model, therefore, the membrane potential following a spike returns to that level which would be predicted by the ongoing synaptic activity without a spike, although the spike threshold is transiently increased.

The responses of this model to various random and deterministic inputs have been studied on a digital computer. We have confirmed the observation of Segundo, et al. (Kybernetik 5, 157-171) that non-resetting models readily produce high-frequency bursts, a property characteristic of neurons in the cuneate nucleus. The cross-correlogram of resetting models has been reported to be similar to the time derivative of the EPSP (Knox, Biophys. J. 14, 567-582). In our model, however, the cross-correlogram is closer in shape and time course to the EPSP itself.

We are now performing a quantitative study of the influence of several "afferent" (e.g., afferent input statistics, EPSP amplitude, and synaptic noise level) and intrinsic "cell" variables (e.g., membrane time constant, degree of post-spike refractoriness, recurrent IPSP's) upon the interspike interval histogram, joint interval distribution, auto- and cross-correlograms, and the power spectrum and cross-spectrum.

182.9 A COMPUTER SYSTEM FOR SEMI-AUTOMATIC CELL RECOGNITION IN NEUROANATOMIC STUDIES. D.S. Schlusselberg, W.K. Smith, B.G. Culter and D.J. Woodward. Department of Cell Biology, University of Texas Health Science Center, Dallas, TX 75235.

A common task in neuroanatomical research is the identification of specifically stained cells in sections of brain tissue examined with a light microscope. Positions of these cells are commonly plotted in relation to cytoarchitectonic boundaries and brain landmarks, and then displayed as sequences of serial sections. A computer data acquistion system has been developed to store this anatomical information in disk files, allowing quantification and viewing of cell distributions in a three-dimensional form. The most time consuming aspect of the current version of this system is the manual entry of cell positions, especially in studies where large volumes of tissue are involved. We report here that significant progress has been made toward developing a general set of strategies for automating the visual recognition process by the use of a high speed graphics processor which analyzes digitized video images obtained from a light microscope.

A host computer controls stepper motors on a light microscope stage to scan a user-defined region of a given section. This region is divided into scanning squares which are sequentially digitized into a video frame buffer and analyzed by a high speed microprogrammable graphics processor. The cell recognition program scans the digitized image for shades above or below a certain intensity threshold, and subsequently identifies the region bounded by pixels outside the threshold shade range. If the area of this region is within a window of user-defined values the region is accepted as a cell, and its center, area and optical density are stored in local memory. A cross appears in the video image over regions selected as cells. The algorithm counts objects in a 512 by 512 pixel array in about one-third of a second, allowing program values and microscope lighting to be adjusted interactively to achieve the optimal accuracy of cell recognition. After selection of these parameters the user can add or delete cells, and then signal the program to store the list of cell descriptions obtained from a single field scan. Cell locations are stored as biological coordinates defined by a zero point and axis for each section. Cells whose centroids are near scan box boundaries are identified and flagged so that they are only counted once in subsequent scanning fields. The initial version of the system is being applied to the analysis of pigmented dopamine cells in human brainstems, and HRP-filled projection cells in rat thalamus and cortex. The raster graphics hardware employed for image analysis can also be used to create three-dimensional views of cell distributions. (Support from DA-2338, AA-0390 and the Biological Humanics Foundation.)

183.1 STANDARDIZATION OF ^{3}H-TISSUE IMAGES WITH DIFFERENT ISOTOPIC STANDARDS, T.W. Lysz, A.W. Toga and W.A. Geary II. Dept. of Pharmacol. and Neurol., Washington Univ. Sch. of Med., St. Louis, MO 63110.

Quantitative autoradiography requires the ability to standardize the optical density (OD) of an image to a known concentration of radioactivity in tissue. Until recently, ^{3}H-images have been standardized using ^{14}C-standard plastics. We have compared the standardization of ^{3}H-images using ^{3}H- and ^{14}C-plastic standards.

Tissue ^{3}H-images were produced by injecting 6 rats with variable doses of ^{3}H-2-deoxyglucose (100-1000 µCi). After 1 hour, brains were rapidly perfused, fixed, and hemisected. One half brain was dissected into thalamus, striatum, and frontal cortex. The other half brain was frozen, mounted, and processed for film autoradiography. Dissected tissues were homogenized and treated with 1N NaOH for 2 hours at 80°C. Triplicate samples were neutralized and counted by liquid scintillation spectroscopy. Sections for autoradiography were cut at 20 µm at -20°C and exposed for 12 days against LKB Ultrofilm at room temperature. ^{3}H-containing plastic was made by adding variable amounts of ^{3}H to 1 ml of liquid methacrylate monomer and subsequent mixing with 2 grams of methacrylate polymer. After a 12 day exposure, a tissue OD vs tissue nCi/mg curve was generated with a digital mini-computer using a program for the best fit polynomial regression by the method of least squares. ^{3}H- and ^{14}C-plastics were calibrated by interpolation.

To examine the relationship between exposure time and resultant OD, calibrated ^{3}H- and ^{14}C-plastic standards were exposed against LKB Ultrofilm for 7, 12, and 20 days. Both isotopes produced lower optical density with shorter exposure time and increased OD with longer exposure time. However, the effects of variable time of exposure was not the same for the two isotopes. For a tissue radioactivity of 5 nCi/mg, calibrated ^{3}H-plastic displayed ODs of 0.34, 0.40, and 0.68 for 7, 12, and 20 days, respectively. ^{14}C-standard plastic calibrated at 5 nCi/mg produced 0.13, 0.40, and 1.05 ODs for the three exposure times. Equivalent ODs for ^{3}H- and ^{14}C-plastics at 12 days result from the original 12 day calibration exposure. The results show a lack of covariation between ^{3}H- and ^{14}C-generated images with changing exposure times. Thus, the use of ^{14}C-isotope to standardize ^{3}H-tissue images requires either strict adherence to a single exposure time or the generation of multiple calibration curves for different exposure times. The use of ^{3}H-plastic for ^{3}H-tissue images circumvents this problem.

183.2 QUANTITATIVE AUTORADIOGRAPHY OF OPIATE BINDING SITES IN RAT CNS. W.A. Geary II and G.F. Wooten, Depts. of Pharmacol. and Neurol., Washington Univ. Sch. of Med., St. Louis, MO 63110

We have employed quantitative autoradiography of radioligand binding in fresh frozen brain sections (Sci. 214, 1981) to describe opiate binding sites in the rat CNS. This abstract describes the method and characterizes the pharmacology of ^{3}H-naloxone (45 Ci/mmole) and ^{3}H-etorphine (35 Ci/mmole) binding to brain sections.

Rats were sacrificed by decapitation and brains were rapidly removed, frozen, and prepared for frozen sectioning. Serial 20µm sections at the level of the striatum were cut coronally and stored at -35°C until needed. For experimentation, sections were dried at 20°C and preincubated for 5 minutes in iced PBS (pH 7.4). After preincubation to remove endogenous ligands, sections were incubated in PBS buffer containing radioligand for 30 minutes at 20°C (total binding). Preliminary experiments showed equilibrium conditions at 30 minutes. Nonspecific binding was determined by incubating matched adjacent sections in buffer containing radioligand plus excess (200 nM Naloxone-HCl or 150 nM Etorphine-HCl) unlabeled drug. After incubation to equilibrium, sections were rapidly rinsed (3-5 sec) twice in iced buffer and once in iced deionized H_2O. After air drying at 20°C, sections were co-exposed with radioactive plastic standards for 12 days to LKB Ultrofilm. For initial saturation studies, sections were also solubilized and counted by liquid scintillation spectroscopy.

Analysis of solubilized brain sections showed saturable binding for both ^{3}H-naloxone and ^{3}H-etorphine. Scatchard analysis revealed K_D = 1.42 nM and B_{max} = 38.2 fmoles/section for the antagonist and K_D = 1.03 nM and B_{max} = 75.1 fmoles/section for the agonist.

Quantitative densitometry yielded Scatchard analyses in close agreement with scintillation spectroscopy: K_D = 1.55 nM and B_{max} = 27.2 fmoles/mg tissue wet weight for ^{3}H-naloxone and K_D = 1.47 nM and B_{max} = 71.1 fmoles/mg tissue wet weight for ^{3}H-etorphine for the striatum.

Pharmacological specificity was determined by competition experiments using 2.0 nM ^{3}H-naloxone and several opiate drugs. Stereospecificity was demonstrated by a levorphanol IC_{50} of 5.5 nM while dextrophan showed an IC_{50} >1000 nM. The rank order potency for competition with 2.0 nM ^{3}H-naloxone was as follows: etorphine (IC_{50} = 0.9 nM) > naloxone (IC_{50} = 4.0 nM) > levorphanol (IC_{50} = 5.5 nM) > morphine (IC_{50} = 125 nM) > dextrorphan (IC_{50} = 1000 nM). The distribution of binding sites was in agreement with published descriptions of opiate binding sites in the rat CNS. The use of quantitative autoradiography will facilitate the regional characterization of opiate binding sites.

183.3 QUANTITATIVE AUTORADIOGRAPHY OF ^{3}H-SPIPERONE BINDING SITES IN THE RAT STRIATUM. G.F. Wooten, W.A. Geary II and M.B. Ferrari*. Depts. of Pharmacol. and Neurol., Washington Univ. Sch. Med., St. Louis, MO 63110.

Some of the biochemical properties of ^{3}H-spiperone binding to rat striatum were studied by quantitative in vitro autoradiography (Eur. J. Pharmacol. 72:421-422, 1981 and Neurosci. Letters 25:101-105, 1981).

Following decapitation, brains were rapidly removed, frozen in liquid freon, and mounted on brass chucks. Serial 20 µ sections were taken in the coronal plane through the striatum and stored at -35°C for up to 10 days. Prior to binding studies the sections were thawed to 20°C and preincubated for 5 min. in iced PBS (pH 7.4). Because preliminary studies showed that equilibrium was reached in approximately 20 minutes at room temperature, incubations were carried out for 30 minutes. Nonspecific binding was determined by incubating adjacent sections in buffer containing radioligand plus d-butaclamol 1 µM (l-butaclamol 1 µM had no effect on binding). Following incubation for 30 minutes at room temperature, sections were rinsed for five seconds x2 in iced PBS and once in iced deionized water. After drying at room temperature, sections were exposed along with plastic standards to LKB Ultrofilm for 12 days. After development, the film was analyzed densitometrically with a Leitz MPV Microdot variable aperture microdensitometer.

There was no effect of tissue thickness between 5 and 40 µ on the autoradiographic images. Saturation curves for ^{3}H-spiperone, when subjected to Scatchard analysis, revealed a Kd = 0.45 nM and a Bm = 48 fmoles/mg tissue in the striatum. Striatal binding was stereospecific as the IC_{50} for d-butaclamol was approximately 8 nM while the IC_{50} for l-butaclamol was 10 µM (^{3}H-spiperone concentration was 1 nM). The pharmacologic specificity of ^{3}H-spiperone binding was characterized by competition experiments for binding against ^{3}H-spiperone 1 nM. The following IC_{50}'s were determined: fluphenazine < 0.1 nM, spiperone 0.8 nM, haloperidol 15 nM, apomorphine 0.2 µM, dopamine 20 µM, and serotonin 110 µM.

In studies of regional ^{3}H-spiperone binding by quantitative autoradiography, there was a highly significant ($P < 0.001$) correlation between regional in vitro binding and the regional cerebral distribution of ^{3}H-spiperone determined autoradiographically 2 hours after an intravenous injection of ^{3}H-spiperone in vivo.

The use of quantitative in vitro autoradiography will greatly facilitate the biochemical characterization of regionally distinct drug and neurotransmitter binding sites.

183.4 BENZODIAZEPINE RECEPTORS: AUTORADIOGRAPHIC LOCALIZATION IN RAT AND HUMAN AMYGDALA. D.L. Niehoff and M.J. Kuhar. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Balto., MD 21205

Electroencephalographic, evoked potential and behavioral studies have implicated the amygdaloid nucleus as an important site of action of benzodiazepine (BZ) drugs. Within this structure, the lateral, basal, and central nuclei, and the anterior rather than the posterior aspect have been demonstrated to be responsive to BZ's by means of studies involving lesions of these nuclei or direct application of BZs. As expected, light microscopic autoradiography (LM-ARG) has demonstrated a high density of BZ receptors in the amygdala. The triazolopyridazine drugs (TPZ's), typified by CL218,872, appear to differentiate two subtypes of BZ receptors, Type 1 (TPZ-preferring) and Type 2 (TPZ non- preferring). In this study, we have utilized a LM-ARG technique involving CL218,872 (Young et al., J. Pharmacol. Exp. Ther. 216:825, 1981) to localize multiple BZ receptors in rat and human amygdala and to correlate these results with previous data on BZ effects on this structure.

Multiple BZ receptors in 8 micron rat or human slide-mounted tissue sections encompassing the entire rostral-caudal extent of the amygdala were labeled according to Young et al., 1981. Autoradiograms were generated by the apposition of emulsion-coated coverslips.

In the rat, the highest density of BZ receptors was found in the lateral nucleus, and the lowest in the central nucleus. Both anterior and poterior cortical nucleus and the more caudal portions of the basolateral and basomedial nuclei also contained high levels of receptors. At all levels, the majority of the receptors appeared to be of the Type 2 subtype, except in the anterior aspect of the lateral nucleus and the most anterior portion of the anterior cortical nucleus. Thus, these results can be correlated with previous results suggesting the importance of the lateral nucleus and the anterior aspect of the amygdala in the action of BZ's.

Because the human brain has been shown to differ from that of the rat in both the gross distribution of BZ receptors and the degree of heterogeneity in several regions, the distribution of multiple BZ receptors in human amygdala will be compared to that in the rat. Species differences may have implications for the development of animal models for human anxiety. An attempt will be made to identify specific neuronal circuits that are likely to be affected by BZ administration.

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183.5 AUTORADIOGRAPHIC STUDIES ON GABA$_A$ RECEPTOR SITES IN THE RAT BRAIN. S. Kito, E. Itoga*, Y. Nakamura* and T. Kishida*. Third Dept. of Int. Med. Hiroshima Univ. School of Med., Hiroshima, Japan 734.

γ-Amino butyric acid (GABA) receptor sites are widely distributed in the mammalian central nervous system. Recently, GABA receptor sites were classified into GABA$_A$ sites and GABA$_B$ sites according to bicuculline sensitivity, i.e. GABA$_A$ sites were bicucullin sensitive and revealed a high affinity for muscimol whereas GABA$_B$ sites were bicucullin insensitive and had a high affinity for baclofen (Bowery 1980). We investigated GABA$_A$ receptor sites in the rat cerebellum through biochemical binding experiments using cryostat sections. We also observed autoradiographic distribution of GABA$_A$ sites in the rat brain with use of ^{3}H-GABA as a ligand. Wistar strain male rats were used for the experiments. The cerebellum was obtained by decapitation and 10 um frozen sections were made by cryostat. The sections were stored at -20°C and used within a week. For the binding assay, slides were preincubated in 50 mM Tris HCl buffer containing 2 mM CaCl$_2$ (pH 7.4) for 20 min at 20°C. Then the slides were incubated with various concentrations of ^{3}H-GABA and 50 uM baclofen (to suppress the ^{3}H-GABA binding to GABA$_B$ receptor sites) with or without 0.2 mM GABA. After the incubation, the slides were rinsed in the same redioactive-free buffer and the tissues were wiped away with Whatman filter paper, placed in a scintillation vial and disolved with 1 ml Soluene. Twelve hours later, the scintillation fluid was added to the samples and counted. Scatchard analysis of specific ^{3}H-GABA binding to GABA$_A$ sites in cerebellar cryostat sections indicated the presence of saturable binding with two components. Namely, one component with the Kd value of 9.8 nM and the Bmax of 274 fmol/2 sections and another component with the Kd value of 0.16nM and the Bmax of 55 fmol/2 sections were determined. The Kd value obtained from kinetic experiments was 13 nM. As the next step, we tried to observe distributions of these high and low components of GABA$_A$ receptor sites in the rat brain autoradiographically. Incubation of cryostat sections with 0.2 nM and 10 nM ^{3}H-GABA with 50 uM baclofen followed preincubation. The slides were rinsed, air dried, carbon coated and coated with nuclear track emulsion. After 8 week exposure, the slides were developed and stained with toluidine blue. The distribution pattern of both components of the GABA$_A$ receptor site was similar to that of ^{3}H-muscimol binding sites in the cerebellam. However,in the striatum and substantia nigra, binding sites of the low affinity component were more mumerous than in other regions of the brain. These distributions were visualized and quantified by the Texture Analyzing System.

183.6 IMPLICATIONS OF TRANS-SYNAPTIC DEGENERATION FOR RECEPTOR BINDING STUDIES IN THE RAT STRIATUM. T. Hattori, P. Weinreich* and P. Seeman (SPON: J.I. Nagy). Dept. Anat. and Pharmacol., Med. Sci. Bldg., Univ. Toronto, Toronto, Ont. Canada

The decrease of the number of striatal dopamine receptors after decortication has been interpreted to indicate that these receptors are contained on terminals of the corticostriatal projection and are therefore presynaptic. However, in view of the total absence of classical axo-axonic synaptic contacts between two boutons in this brain area, other interpretations should be investigated. We now suggest that trans-synaptic degeneration of striatal dendritic spines which receive synaptic inputs from both the corticostriatal and nigrostriatal projections may explain the loss of receptor binding. Two days after decortication, ^{3}H-spiperone radioceptor assays revealed approximately a 15% decrease in binding, although this value was not significant due to the large variability between animals. Maximal and uniform reduction in binding occurred at 5 days postlesion (45% decrease) with no further change apparent up to 19 days postlesion. Two and three days postlesion, degenerating dendritic spines that were postsynaptic to degenerating boutons were often observed. This evidence ranged from examples of a degenerating bouton in synaptic contact with a normal appearing spine (both of which were totally engulfed by astrocytes) to the most advanced stage where both the terminal and postsynaptic spine showed dark type degeneration. At 3 days postlesion, out of 750 cases of degeneration, about 33% were in the final stage. The present data clearly indicate that trans-synaptic degeneration occurs in the striatum. Therefore, it cannot be concluded that a decrease in the number of receptors after cortical lesions necessarily indicates that these receptors are localized on the presynaptic terminals. It is equally possible that this reflects a loss of receptors that were located on dendritic spines that underwent trans-synaptic degeneration.

183.7 D-LSD BINDING IN MOUSE BRAIN: DIFFERENCES BETWEEN IN VIVO AND IN VITRO LOCALIZATION. B.J.Ebersole*, S.Maayani, R.Weiśbach and H.Weinstein*. Department of Pharmacology, Mount Sinai School of Medicine of CUNY, New York, NY, 10029.

Establishing a relationship between binding of [^{3}H]-d-lysergic acid diethylamide([^{3}H]LSD) and its pharmacology requires consideration of dynamic mechanisms that may modulate interactions of the drug with the binding sites. Such mechanisms may cause differences between the characteristics of LSD binding measured in equilibrium in vitro and non-equilibrium in vivo experiments. Using radioautographic localization and high affinity binding to mouse brain membranes, we found differences between the localization of [^{3}H]LSD in vitro and following in vivo administration of the drug. Mice injected i.v. with 100nmol [^{3}H]LSD/kg, with or without coinjection of 5µmol/kg unlabeled LSD, were killed 15 min later,when brain drug levels are maximal. Brains were dissected, homogenized, filtered, and rinsed with cold buffer. [^{3}H] bound to membranes was unchanged LSD. [^{3}H]LSD conc. in all regions was ca. 12nmol/kg tissue. In animals receiving excess LSD, bound [^{3}H]LSD in cerebellum was not reduced; bound [^{3}H]LSD in both cortex and hippocampus was reduced to cerebellar levels."Specific" binding was thus defined for each animal as binding in excess of cerebellar levels.

	Total bound	"Specific" bound	Levels are
CTX	4.5 + 0.6	3.6 + 0.5	expressed as nmol
HIP	1.8 + 0.2	0.9 + 0.2	[^{3}H]LSD/kg tissue,
CER	0.9 + 0.2	-------	mean+SEM, n=6

Specific binding in HIP is significantly less than in CTX. In contrast, specific [^{3}H]LSD binding in vitro (defined in the absence and presence of 1 uM LSD) was similar in CTX and HIP with half-maximal saturation at 5nM and B_{max}=200 fmol/mg protein. The in vivo/in vitro difference in the ratio of HIP:CTX specific binding was confirmed by radioautographic studies of brain slices exposed to [^{3}H]LSD under in vivo(as above) or in vitro(6nM [^{3}H]LSD) conditions. Labeling in CTX was similar under both conditions,with a dense band seen in both layers III-IV of CTX and anterior cingulate gyrus. This band was designated "specific" labeling as it was not apparent in competiton experiments with unlabeled LSD (1uM in vitro,5µmol/kg injection in vivo). In contrast, marked differences were seen in HIP. In vitro, specific label was seen in all areas except CA2 and CA3. In vivo, the slight label--seen in CA1, CA2, CA3, and CA4, but not in dentate gyrus--was not affected by co-injection of excess LSD, indicating a lack of specific binding. Ongoing studies aim to determine if the observed differences are due to effects of endogenous modulators or neurotransmitters, or to time and/or region-dependent modes of interaction of the drug, as determined by pharmacokinetic or pharmacodynamic characteristics. [Supported by USPHS Grants DA-01875 and DA-00060,and Predoctoral Training Grant GM07163(B.J.E.)]

183.8 REGIONAL DIFFERENCES OF 2-BROMO D-LSD AND D-LSD INTERACTION WITH 5-HT AND LSD BINDING SITES. S. G. Beck, S. Maayani, H. Weinstein*, and J. P. Green. Department of Pharmacology, Mount Sinai School of Medicine of CUNY, New York, NY 10029.

LSD (d-lysergic acid diethylamide) produces hallucinations in man, but its 2-bromo derivative (BOL) does not. However, other physiological and mental effects are elicited by both drugs, but BOL is 20-100 fold less potent. Delineation of pharmacological mechanisms responsible for such differences between related drugs should help to elucidate the mode of action of LSD in brain. We used high affinity binding methods and radioautographic localization to compare the interaction of LSD and BOL with specific binding sites in various regions of rat brain. Competition of LSD and BOL for binding sites in P_2 membrane preparations from female rat cortex (CX) or hippocampus (HIP) was studied with several concentrations of [^{3}H]LSD or [^{3}H]5-HT. [^{3}H]LSD was used at 2 and 6nM and [^{3}H]5-HT was used at 0.5, 2 and 6nM, concentrations chosen to occupy 50-80% of available sites. Non-specific binding was defined with 10^{-6}M unlabeled LSD. In CX, BOL and LSD appeared equipotent at both [^{3}H]LSD concentrations with Hill coeffs. near unity. However, BOL appeared four fold less potent than LSD at all [^{3}H]5-HT concentrations. Competition by BOL deviated from simple competitive behavior at the higher concentrations but not at 0.5nM. Hill coeffs. for competitions with the increasing concentrations of [^{3}H]5-HT were 0.93, 0.86, and 0.64 respectively. In contrast, Hill coeffs. for competition by LSD deviated from unity (0.82) only with 6nM [^{3}H]5-HT. Differrences between LSD and BOL were greatest in HIP where BOL was four fold less potent than LSD at both concentrations of [^{3}H]LSD. Hill coeffs. for both LSD and BOL were unity. These results are consistent with the hypothesis that BOL and LSD interact with at least two populations of serotonin binding sites labeled by both [^{3}H]LSD and [^{3}H]5-HT. The difference in the affinity of BOL for these populations is much greater than that of LSD. That such differences between BOL and LSD were more apparent in HIP than in CX is consistent with previous findings of a regional heterogeneity in the distribution of multiple serotonin sites. In CX the sizes of the populations appear equal, whereas in HIP one population predominates. This regional difference between BOL and LSD was confirmed by results from in vitro radioautography experiments with [^{3}H]LSD and [^{3}H]5-HT. Consistent with results from high affinity binding in homogenates, BOL (10^{-6}M), like LSD, eliminated the binding by [^{3}H]LSD in CX, but did not eliminate the labeling of HIP by [^{3}H]LSD or [^{3}H]5-HT. This pattern of selective regional competition for sites labeled by [^{3}H]LSD and [^{3}H]5-HT is identical to the one observed in our radioautography studies with the neuroleptic spiroperidol. (Supported by USPHS Grants DA-01875, DA-00060 and DA-07135)

183.9 CHARACTERIZATION OF MULTIPLE [^{3}H]5-HYDROXYTRYPTAMINE BINDING SITES IN RAT SPINAL CORD TISSUE, P.J. Monroe & D.J. Smith, Dept. of Pharmacology/Toxicology & Anesthesiology, W.V.U. Medical Center, Morgantown, West Virginia 26506.

Multiple serotonin (5HT) binding sites apparently exist in several areas of the central nervous system (Snyder & Goodman, J. Neurochem. 35: 5, 1980). For example, Peroutka & Snyder (Mol. Pharmacol. 16: 687, 1979) reported two sites in the rat frontal cortex, one of which ($5HT_1$) has a high affinity for ^{3}H-5HT while the other binds ^{3}H-spiperone preferentially. In the present study the existence of a similar multiple 5-HT binding site system was studied in tissue from the rat spinal cord.

A membrane fraction was prepared from spinal cord tissue by the method of Peroutka & Snyder (see reference). Binding assays for ^{3}H-5HT were carried out with these membranes at 37°C for 20 min. using 800 µl of tissue suspension (20 mg tissue/tube), 100 µl of ^{3}H-5HT and 100 µl of drug. After the incubation, the tissue was collected on Whatman GF/B filters and radioactivity analyzed in Dimilume after a 5 hr extraction (spec./total binding=35-40%). Binding assays using ^{3}H-spiperone as the radioligand were also performed. Some assays were conducted with each radioligand in frontal cortex tissue for comparison to the spinal cord data.

The results indicated that multiple 5-HT binding sites exist in the spinal cord. A curvilinear Scatchard plot results from saturation studies with [^{3}H] 5-HT. Two apparent sites are resolved with dissociation constants of 6.7 & 33.0 nM for the high and low affinity sites, respectively. Additional support for the existence of multiple 5HT binding sites comes from the analysis of the displacement of 30 nM ^{3}H-5HT by the unlabeled ligand, where Hill coefficients (n_H) were significantly less than unity (0.32). Similarly, Hill plot analysis indicates that unlabeled spiperone interacts at these multiple 5HT sites (n_H=0.50). However, specific ^{3}H-spiperone binding, which can be demonstrated in the frontal cortex is absent in the spinal cord. None of the specifically bound ^{3}H-5HT was found to be associated with the 5HT uptake carrier since fluoxetine, an inhibitor of 5HT uptake, does not alter binding characteristics.

The high affinity spinal cord site is apparently analogous to the high affinity $5HT_1$ binding site located in the frontal cortex. A dissociation constant similar to that for the high affinity spinal cord site can be calculated from saturation studies using frontal cortex tissue (K_D=4.4 nM), and in both tissues, spiperone displays nearly equal potency (IC50 value against 2 nM ^{3}H-5HT in frontal cortex, 3µM, and spinal cord, 6 µM). However, the low affinity site in the spinal cord is not analogous to the $5HT_2$ site in frontal cortex that preferentially binds ^{3}H-spiperone.

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183.10 NEUROTENSIN RECEPTORS AND OPIATE RECEPTORS IN THE FOREBRAIN OF THE RHESUS MONKEY: AUTORADIOGRAPHIC LOCALIZATION. Miles Herkenham, Steven P. Wise, Remi Quirion, and Candace B. Pert. Laboratory of Neurophysiology and Biological Psychiatry Branch, NIMH, Bethesda, Maryland 20205.

Previous studies have emphasized the similarities between mu-opiate and neurotensin receptor distributions. Accordingly, these receptors were visualized autoradiographically in slide-mounted sections through the forebrain of a rhesus monkey. The ligands used to label the receptors were [^{3}H]naloxone (NAL) and [^{3}H]neurotensin (NT), respectively (see Herkenham and Pert, J. Neurosci., in press; Young and Kuhar, Brain Res., 1981, 206: 273).

Cerebral cortex. The patterns of NT and NAL binding in the cerebral cortex are stikingly similar. In general, deep cortical layers (layers V and VI) show the highest levels of binding. This distribution suggests that the output elements of cortex may be most subject to neurotensinergic and opiatergic modulation. For both receptors, cortical fields in proximity to the allocortex are receptor-dense while the classical visual, somatosensory and motor fields are receptor-sparse. Thus, both of these receptors are preferentially distributed in limbic cortex, specifically the cingulate, entorhinal, parasubicular, insular, prostriate, orbital frontal, and medial frontal cortex. While we note the marked enrichment of opiate receptors in the limbic cortex relative to the sensory and motor fields, this disparity is even greater for NT receptors.

Striatum. NT and NAL strongly contrast in their binding to the striatum. NAL binding to striatal opiate receptors is distributed densely and heterogeneously in the ventromedial striatum and displays a gradient of decreasing density towards the dorsolateral striatum. NT has low and homogeneous binding levels in the caudate and putamen and moderate binding in the lateral portions of accumbens nucleus. Thus, the striatal binding patterns indicate a significant exception to the general correspondence of opiate and NT receptors.

Conclusions. The di- and telencephalic structures most heavily labeled by NAL include most of the amygdala, many parts of thalamus and hypothalamus, the septal nuclei, the ventromedial striatum, and the basal nucleus of Meynert. Of these, only the basal nucleus is heavily labeled by both NT and NAL. By contrast, NT most heavily labels the endopiriform nucleus, parasubiculum, CA_4 hippocampus, and the limbic iso- and juxtallocortex. Hence, most of the NT receptor-dense regions are within cortex, and more specifically, limbic cortex. In view of their more limited localization to limbic forebrain structures, NT receptors may have a more circumscribed role in brain function than do opiate receptors.

183.11 RADIOHISTOCHEMICAL LOCALIZATION OF OPIATE RECEPTORS IN RAT BRAIN: THE BENZOMORPHAN BINDING SITE. F. Valdes*, B. Crain, K. Chang, and J. McNamara (SPON: R. Dasheiff). Departments of Medicine, Pathology, and Pharmacology, Duke University Medical Center and The Wellcome Research Laboratories, Research Triangle Park, Durham, NC 27705.

Recent biochemical investigations discovered the presence of a subpopulation of opiate receptor binding sites with high affinity for several benzomorphan drugs (PNAS 78:4141, 1981). These sites exhibit high affinity for opiate receptor antagonists (e.g. diprenorphine) and low affinity for both mu (e.g. morphiceptin) and delta (e.g. [D-Ala^2, D-Leu^5] enkephalin) agonists. These sites have therefore been termed benzomorphan binding (BB) sites. Elucidating the functional significance of these binding sites will be facilitated by understanding their anatomic distribution. We therefore analyzed the distribution of BB sites with the in vitro radiohistochemical method of Young and Kuhar.

Thin (10 micron) frozen sections of rat brain were thaw mounted on gelatin coated slides. To promote removal of endogenous opioids, the sections were preincubated in 50 mM Tris Cl buffer pH 7.7 containing 50 µM GTP and 100 mM NaCl. The sections were then incubated at 25° C for 40 min. in 170 mM Tris Cl buffer ph 7.7 containing 2 nM [^{3}H] diprenorphine (DPN) under four different conditions: 1) [^{3}H] DPN alone (labelled mu, delta and BB sites); 2) with added 10^{-5} M morphiceptin (labelled delta and BB sites); 3) with added 10^{-5} M morphiceptin + 2 X 10^{-7} M [D-Ala^2, D-Leu^5] enkephalin (labelled BB sites); or 4) with added 2 X 10^{-6} M DPN (nonspecific). The sections were rinsed, dried, and apposed to either emulsion (Kodak NTB2) coated coverslips or tritium sensitive film (LKB ultrofilm [^{3}H]).

The BB sites were preferentially enriched in gray matter compared to white matter. BB sites were found in the following areas (density ranging from the highest to the lowest): amygdala = hypothalamus > cortex = caudate > thalamus = hippocampal formation. Areas particularly enriched were the habenula, the most dorsal medial portion of caudate, and the interstitial nucleus of the stria terminalis. These findings should provide a framework for pursuing sites at which the various pharmacologic actions of the benzomorphans are mediated.

183.12 CHARACTERIZATION OF THE AFFINITY PURIFIED GLYCINE RECEPTOR OF RAT SPINAL CORD. F. Pfeiffer*, D. Graham* and H. Betz* (SPON: ENA). Max-Planck-Institute for Psychiatry, Department of Neurochemistry, 8033 Martinsried, Federal Republic of Germany.

Glycine is a major inhibitory neurotransmitter in mammalian spinal cord. The hyperpolarizing action of this amino acid can be selectively antagonized by the alkaloid strychnine, the latter being a highly specific ligand for the glycine receptor. Recently, this receptor has been solubilized from rat spinal cord membranes using the non-ionic detergent Triton X-100 (Pfeiffer and Betz, Brain Res. 226: 273, 1981).

The glycine receptor was purified 1950-fold by affinity chromatography on aminostrychnine-agarose and wheat germ agglutinin-Sepharose. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and mercaptoethanol revealed three glycine receptor associated polypeptides of molecular weight 48,000, 58,000 and 93,000. Using our previously published photoaffinity-labeling procedure (Graham et al., Biochem. Biophys. Res. Comm. 102:1330, 1981), ^{3}H-strychnine was incorporated irreversibly into the 48,000-dalton polypeptide. The dissociation constant of ^{3}H-strychnine binding to the purified glycine receptor was 9.3 nM. The glycine receptor agonists glycine, ß-alanine and taurine inhibited the binding of ^{3}H-strychnine. Gel filtration and sedimentation in sucrose/H_2O and sucrose/D_2O gradients gave a Stokes radius of 7.7 nM, a partial specific volume of 0.78 ml/g and a sedimentation coefficient of 8.2 S for the purified glycine receptor. From these data, a molecular weight of 246,000 was calculated for the glycine receptor protein.

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183.13 DOPAMINE RECEPTOR TURNOVER AFTER VIPOXIN BLOCKADE. Jonathan E. Freedman and Solomon H. Snyder. Johns Hopkins University School of Medicine, Depts. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

Vipoxin is a small, basic protein, molecular weight 13,000, which we have purified to apparent homogeneity from Russell's viper venom. Vipoxin inhibits ^{3}H-ligand binding to alpha-adrenergic, dopaminergic, and serotonergic receptors. The inhibition of binding is highly selective and potent, and essentially irreversible (J. Biol. Chem. 256, 13172).

We have studied effects on dopamine receptors after stereotaxic injection of vipoxin into the corpus striatum of pentobarbital-anesthetized rats. Vipoxin reduces ^{3}H-spiroperidol binding to dopamine receptors in striatum homogenates, with 5 µg of vipoxin inducing a 50% reduction in binding 5 hr after injection. The reduction in binding reflects a decrease in the number of receptor sites rather than a change in binding affinity. The rats also display rotational stereotopy ipsilateral to the injection site. Vipoxin does not reduce ligand binding in brain regions other than the region injected. ^{3}H-Spiroperidol binding sites begin to reappear within 24 hr after injection, and return to control values at around 2 days. The kinetics of reappearance can be described by receptor synthesis at the rate of approximately 40 fmol/mg protein-hr and receptor degradation with a rate constant of 0.06 hr^{-1}, corresponding to a receptor half-life of approximately 12 hr.

Because vipoxin also acts at alpha-adrenergic and serotonin receptors, this technique can also be applied to these receptors in other brain regions. Furthermore it is possible to determine the relative effects on receptor synthesis and receptor degradation giving rise to such changes in receptor number as the supersensitivity of dopamine receptors after chronic neuroleptic treatment or nigrostriatal lesion.

183.14 [^{3}H]N-ACETYLSEROTONIN LABELS SEROTONERGIC RECEPTORS IN RAT FRONTAL CORTEX. L. P. Niles, G. M. Brown and R.K. Mishra* Department of Neurosciences, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

In attempting to determine how the pineal gland influences brain and endocrine function in mammals we have examined binding of the putative pineal hormone N-acetylserotonin in CNS tissues. Using tritiated N-acetylserotonin ([^{3}H]NAS), saturable, high affinity binding was found in human, rat and calf brain membranes. In fresh rat brain membranes, suspended in 50mM tris Hcℓ, pH 7.4, [^{3}H]NAS binding was monophasic with dissociation constant (K_D) values ranging from 3 - 7 nM and a binding site concentration (Bmax) of 230 - 400 fmol/mg protein. Frozen membranes displayed biphasic scatchard plots with a high affinity binding component similar to that in fresh tissue and a second population of low affinity, high capacity binding sites.

TABLE 1

	Ic_{50}(nM)	nH
NAS	10.89	1.09
5-HT	19.59	1.21
5-Methoxytryptamine	432	0.82
N-acetyltryptamine	$>10^6$	
Quipazine	190	0.49
Methysergide	83	0.86
Mianserine	106	1.01
Ketanserine	118	0.98
Cyproheptadine	5348	0.64
Spiperone	157	0.64

Pharmacological characterization of [^{3}H]NAS binding indicates that the 5-hydroxyl group is necessary for high affinity binding. Thus, among the tryptamines examined, N-acetylserotonin (NAS) and serotonin (5-HT) were the most potent inhibitors of [^{3}H]NAS binding. 5-Methoxytryptamine shows about a 30-fold decrease in affinity, while N-acetyltryptamine, which lacks a C-5 substituent, has negligible effects on binding. The 5-HT agonist, quipazine, and various antagonists exhibited moderate binding affinity. The specific 5-HT_2 antagonist, ketanserine, and spiperone, which reportedly binds to 5-HT_2 sites in the rat frontal cortex, also inhibited binding. These findings suggest that [^{3}H]NAS labels both 5-HT_1 and 5-HT_2 sites in the rat frontal cortex and that the biological effects of NAS may involve interaction at central serotonergic receptors.

(Supported by the Ontario Mental Health Foundation)

183.15 EFFECT OF CHRONIC TREATMENT WITH OVARIAN STEROIDS ON NEUROTRANSMITTER RECEPTORS IN THE RAT BRAIN. A. Biegon*, L. Snyder, and B.S. McEwen. The Rockefeller University, New York, NY 10021.

During the human menstrual cycle or pregnancy in humans and rodents, the brain is exposed to gradually changing and elevated levels of ovarian steroids lasting 2-3 weeks or longer. In order to study the consequences of such hormonal states for neurotransmitter chemistry, we chose to measure the effects of 2-week treatments with estradiol (E_2) and progesterone (P), separately and in combination, on beta and alpha adrenergic and serotonergic receptor levels in rat cerebral cortex. Ovariectomized rats received subcutaneous Silastic implants containing 10% E_2 in cholesterol; pure P; both E_2 and P; or empty capsules. The resulting levels of E_2 approximate proestrous afternoon levels and midpregnancy. Rats were killed two weeks later and cerebral cortex from 5-6 rats was pooled and frozen for subsequent assay of membrane binding of $5HT_1$ (^{3}H 5HT), $5HT_2$ (^{3}H spiperone); beta (^{3}H dihydroalprenolol); $alpha_1$ (^{3}H prazosin) receptors in washed membrane preparations. The experiment was repeated 5-6 times. We found that chronic E_2, P and E_2 plus P significantly reduces $5HT_1$ receptor density (Bmax values in fmoles/mg protein: Control, 864; E_2, 786; P, 732; E_2 plus P, 711; $p < .02$ for all groups compared to control by paired t-test). No significant change in Kd was found. $5HT_2$ receptors increased in density following E_2 (Bmax in fmoles/mg protein: Control, 447; E_2, 581, $p < .01$, paired t-test). A smaller, but still significant increase appears after P alone: Bmax=508 ($p < .05$). E_2 plus P results in no change in $5HT_2$ binding: Bmax=450. $Alpha_1$ adrenergic receptors were unaffected by E_2, P or E_2 plus P treatment. Beta adrenergic receptor density was decreased by chronic E_2, as has been shown previously with a higher level of E_2 (Wagner et al., Brain Res. 71:147, 1979), whereas P had no effect (Bmax in fmoles/mg protein: Control, 608; E_2, 437; P, 513; E_2 plus P, 460, $p < .05$ for paired t-test vs control for E_2 and E_2 plus P, but not for P alone). We conclude that ovarian steroids can modify selectively the densities of serotonergic and adrenergic receptor subtypes. The reduction of $5HT_1$ receptors may be the result of a direct steroid interaction with the membrane (cf., Biegon and McEwen, J. Neurosci. 2:199, 1982). The reduction in beta receptors might be the result of chronic stimulation of norepinephrine release (cf., Wagner et al., cited above). Regardless of mechanism, receptor modulation by steroid hormones may play a role in the affective and behavioral correlates of menstruation and the postpartum period.

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184.1 STRESS INDUCED MODULATION OF HIPPOCAMPAL SYNAPTIC CHOLINERGIC MECHANISMS. Varda H. Gilad, Louis Shenkman, Jose M. Rabey[1] and Gad M. Gilad. Isotope Department, Weizmann Institute of Science, Rehovot, and [1]Ichilov Hospt., Tel-Aviv Univ., Israel.

We have recently demonstrated that inbred Wistar-Kyoto (WKY) rats, which are behaviorally more reactive to stress than Brown-Norway (BN) rats, also have higher choline acetyltransferase (CAT) activity in the septum and hippocampus. And, this is parallelled by a comparable strain difference in acetylcholinesterase staining intensity in the hippocampus. The present study sought to determine whether strain differences also exist in the dynamic regulation of synaptic choline uptake and acetylcholine (ACh) release in the hippocampus after stress. [^{3}H]-choline uptake as measured in synaptosomal preparations (P_2 fractions) was 30% higher in WKY rats, directly correlated to their higher CAT activity. After immobilization stress for 2h, choline uptake was reduced to a similar extent (20 to 30%) in both strains. This reduction in precursor uptake was accompanied by increased ACh release after stress. Release of ACh in control unhandled rats was comparable in both strains. But ACh release after acute stress was 50% higher in WKY than in BN rats. Maximum muscarinic cholinergic binding capacity (Bmax) as assessed by measuring the specific binding of the ligand [^{3}H]-quinuclidinyl benzilate was higher in BN rats (BN:2.216, WKY:1.592pmol/mg protein). The dissociation constant (Kd) however was similar in both strains (BN:5.9×10^{-10}, WKY:6.3×10^{-10}). Binding capacity was not changed after stress. We conclude: 1) Choline uptake in the hippocampus is directly related to CAT activity; 2) After stress,increased ACh release is accompanied by a compensatory reduction in choline uptake; 3) Muscarinic cholinergic binding is inversely related to ACh release after stress, and 4) Reduction in choline uptake may be a hallmark of cholinergic synapses during adaptation to increased neuronal activity.

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184.2 A SODIUM DEPENDENT HIGH AFFINITY CHOLINE UPTAKE SYSTEM UNASSOCIATED WITH ACETYLCHOLINE SYNTHESIS. M. Ivy*, R. Sukumar and J.G. Townsel. Department of Physiology and Biophysics, University of Illinois Medical Center, Chicago, IL 60612.

Low and high affinity choline uptake systems have been demonstrated in neuronal tissues of vertebrates and invertebrates. The low affinity system has been found in diverse tissues and cell types. The high affinity choline uptake system (HACUS), on the other hand, is thought to be restricted in distribution to cholinergic neurons and terminals [Kuhar, M.J. and Murrin, L.C., J. Neurochem. 30: 15-21, 1978]. The HACUS is Na^+ dependent and has been shown to contribute significantly to the synthesis of acetylcholine (ACh) [Yamamura, H.I. and Snyder, S.H., J. Neurochem. 21: 1355-1374, 1973]. In this study we have characterized a sodium dependent HACUS which is exclusive of ACh biosynthesis.

The cardiac ganglion which regulates the neurogenic heart of the horseshoe crab Limulus polyphemus was incubated in Chao's physiological salt solution containing 2 µM [^{3}H] choline at room temperature (25 ± 2°C). The ganglion readily accumulated radioactivity. The rate of uptake was 0.04 p.moles/min/mg tissue. A tissue/medium (T/M) ratio of greater than 7:1 was obtained after 90 min. of incubation in 0.01 µM [^{3}H] choline. The uptake of [^{3}H] choline showed saturation kinetics and was dependent on the presence of sodium ions. Analysis revealed the presence of two kinetically distinct uptake processes for choline - a high affinity uptake system with a Km of 7.92 µM and Vmax of 0.68 p.moles/mg/min and a low affinity uptake system with a Km of 92.37 µM and a Vmax of 9.07 p.moles/mg/min respectively. Neither uptake system was greatly influenced by the absence of calcium and potassium ions in the incubation medium. However, the high affinity choline uptake system was sensitive to some metabolic inhibitors and was significantly blocked by hemicholinium - 3 (HC-3). A comparative study of the [^{3}H] choline uptake products in several neuronal tissues of Limulus including the cardiac ganglion was done. A considerable portion of the label taken up was incorporated into [^{3}H] ACh in the corpora pedunculata (81%), the circumoesophageal ring (57%), and in the abdominal ganglia (47%). In the cardiac ganglion the prevalent product (84%) of the extractable radioactivity taken up co-electrophoresed with phosphorylcholine. [^{3}H] ACh was not detected in extracts of the cardiac ganglion following tissue incubation in either 2 or 5 µM [^{3}H] choline. The ACh synthesizing enzyme, choline acetyltransferase (E.C.2.3.1.6) was also undetected in extracts of the cardiac ganglion. These results suggest that the Na^+ dependent HACUS is not restricted to cholinergic functions.

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184.3 THE EFFECTS OF ANTICONVULSANT DRUGS ON HIGH AFFINITY CHOLINE UPTAKE, J.A. Miller* and J.A. Richter. Departments of Pharmacology and Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

The sedative-hypnotic barbiturate, pentobarbital, when administered in vivo inhibits sodium dependent high affinity choline uptake (HACU) in vitro. Recent studies from this laboratory have suggested, however, that the inhibition of HACU is not correlated with the hypnotic effect (Richter, J.A., et al., J. Neurochem., in press, 1982). We have looked at some other barbiturates and other anticonvulsant drugs to see if there is a relationship between anticonvulsant activity and the inhibition of HACU. In these experiments drugs were administered i.p. to male ICR-Swiss mice and the animals were decapitated at a later time. The hippocampi were removed and HACU was measured in a synaptosomal preparation of this tissue by the method of Simon et al. (Simon, J.R. et al., J. Neurochem. 26: 909, 1976).

The anticonvulsant barbiturates, phenobarbital and barbital, inhibited HACU at doses that did not cause a loss of righting reflex. This further supports the suggestion that the effect of barbiturates on HACU is independent of the hypnotic effect. The brain level of phenobarbital did correlate with the degree of inhibition of HACU. Barbituric acid (which lacks hypnotic or anticonvulsant activity) had no effect on HACU in doses up to 400 mg/kg, supporting the view that the effect of barbiturates is not a nonspecific effect. The benzodiazepine, chlordiazepoxide, also inhibited HACU but at relatively high doses (>18 mg/kg). The anticonvulsant diphenylhydantoin had no inhibitory effect on HACU in doses up to 80 mg/kg when the animals were killed 30 min. postinjection. Thus it appears that the effect of inhibiting HACU may not be common to all anticonvulsant drugs.

In order to characterize further the relationship between anticonvulsant activity of a drug and its ability to depress HACU we plan to correlate the potencies of the drugs as anticonvulsants and as inhibitors of HACU. We also hope to examine interactions of the anticonvulsants with convulsants (e.g., pentylenetetrazole) which can themselves stimulate HACU. It may be that some anticonvulsants may have no effect on HACU unless it is already elevated. (Supported by DA-00796).

184.4 EFFECT OF EXOGENOUS ATP ON $\Delta\Psi$ AND ΔpH IN ISOLATED, CHOLINERGIC SYNAPTIC VESICLES. J.Suszkiw and M. O'Leary Dept. Physiology, Univ. of Cincinnati Medical School Cincinnati, OH 45267

Highly purified (>1500 nmol ATP/mg protein) synaptic vesicles, isolated from the electromotor nerve terminals of Torpedo marmorata, were incubated (30 min,15°) in media consisting of 187.5 mM KSCN,37.5 mM NaCl, 137 mM Lys.Cl,3 mM $MgCl_2$, 0.1 mM $CaCl_2$, 75 mM sucrose and 5 mM HEPES buffer,pH 7.2.

$\Delta\Psi$ and ΔpH were estimated from the distribution of [^{3}H] Ch and [^{14}C]methylamine, respectively, as previously described (J. Suszkiw, in Cholinergic Mechanisms, G. Pepeu and H. Ladinsky, eds. p. 313, Plenum Press, 1981).

In the absence of exogenous ATP, the Ch_{in}/Ch_{out} ratio was 7.7, in agreement with previously determined distribution of [^{3}H]MTPP. Addition of 3 mM ATP to the incubation medium doubled the Ch_{in}/Ch_{out} ratio to 15, indicating hyperpolarization of vesicles from approx. -50 to -68 mV. The effect of exogenous ATP on vesicle membrane potential was observed only in synaptic vesicles that had been previously exposed to Na^+; it was not seen in vesicles that had been isolated in Na^+-free media, and was abolished at 0°. These results provide first demonstration that the vesicle-associated Mg^{+2}-ATPase (1.8 µmol ATP hydrolyzed/30 min./mg protein) may be involved in an electrogenic dissipation of ΔNa^+ (in>out) imposed across vesicle membrane.

The vesicles also accumulate [^{14}C]methylamine. In the absence of ATP the $[^{14}C]MA_{in}/[^{14}C]MA_{out}$ ratio was ∿4; this ratio increased only slightly in the presence of 3mM ATP ($[^{14}C]MA_{in}/[^{14}C]MA_{out}$=5). The corresponding, calculated ΔpHs are 0.6 and 0.7 units, acidic interior. These results indicate that, unlike the Mg^{+2}-ATPase in the catecholaminergic vesicles, the vesicular ATPase does not appear to be involved in the generation of a substantial electrochemical proton gradient in cholinergic synaptic vesicles.

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184.5 CHOLINE EXPOSURE AUGMENTS SUBSEQUENT LOW-AFFINITY CHOLINE ACCUMULATION OF CULTURED HUMAN SKIN FIBROBLASTS. Robert Rybczynski* and Donald Kay Riker, (SPON: W.F. Riker, Jr.) Depts. of Human Genetics and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

In 1981 we reported high-affinity choline accumulation in cultured human skin fibroblasts, defined by an apparent K_T of 3-5μM, hemicholinium-3 sensitivity, and Na^+-dependence (Riker et al, J Neurochem 36:746). We had explored choline concentrations between 0.125-6μM to minimize contributions of low-affinity systems. Extension of this range has now demonstrated both "high" and "low" -affinity components. Further, we report a selective effect of choline, present during the growth phase, on subsequent choline-accumulation kinetics.

Human skin fibroblasts from one of 8 donor lines were plated in 60mm plates in DMEM/5% fetal-calf serum and grown to confluency (7-9 days). Choline, if added, was introduced 24 hrs after plating (final conc.=2mM). All plates were refed 48 hrs before assay. Just prior to assay cells were preincubated (x2) and washed (x4) to remove exogenous choline. Temperature-dependent ^{3}H-choline accumulation was assessed during a 4-min incubation (37° vs. 1°) at choline concentrations of 0.25-35μM. Incubation was stopped with 1° buffer washes (x3), and intracellular tritium counted after lysing cells in 0.6N NaOH.

We constructed Scatchard plots (v vs. v/s) and used Rosenthal's graphic method (Anal Biochem 20:525, 1967) to derive two K_T's and V_{max}'s from curved plots. Plots were curvilinear 60% and linear 40% of the time. Curved plots yielded K_T's of 0.6±0.1SEM and 56±8μM and V_{max}'s of 14.3±1.5SEM and 473±56 pmoles/mg pro/4 min (N=9). These K_T's are similar to those of synaptosomes. Linear plots gave intermediate values (K_T=23.2±6.0; V_{max}= 351±49; N=6). Theoretical argument demonstrates that linear results can be explained by low-affinity "masking" of the high-affinity component. Given the resolution of Scatchard-Rosenthal methods, we conclude that two classes of choline transport, though not always visible, exist in cultured fibroblasts.

By exposing cells to 2mM choline during their growth phase the low-affinity V_{max} increased 327% (473±56 to 2020±116) pmoles/mg pro/4 min), while its affinity decreased 139% (K_T: 56 to 134±62μM). In contrast, the carrier-mediated, high-affinity process did not react to this treatment. At choline concentrations estimated in the extracellular space (4-16μM) such a change would produce an 86-105% increase in the rate of low-affinity choline transport. Thus, previous exposure to choline has the potential to augment its subsequent accumulation by the low-affinity process only.--Supported by the Dystonia Medical Research Foundation, Beverly Hills, CA.

184.6 REGIONAL AND SUBCELLULAR DISTRIBUTION OF ACETYLCHOLINESTERASE ISOENZYMES IN RAT BRAIN. D.M. Maxwell*, D.E. Lenz*, I.J. Dembure* and C.A. Broomfield* (SPON: B. Hackley, Jr.). US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

Recently, we reported that rat cerebral acetylcholinesterase (AChE) exists in four molecular forms distinguished by differences in their pI values and in vivo rates of inhibition by soman, an irreversible inhibitor of AChE (Biochem. Pharmacol., 30:1369, 1981). Since AChE exists in close association with several subcellular components of both muscarinic and nicotinic cholinoreceptors, we undertook to determine whether distribution of these four AChE isoenzymes was related to cholinoreceptor environment or subcellular distribution. The ratio of muscarinic to nicotinic cholinoreceptors in several regions of rat brain were measured using ^{3}H-QNB and ^{125}I-a-bungarotoxin, respectively. The ratio of muscarinic to nicotinic cholinoreceptors in the rat brain regions were: caudate, 67:1, cerebral cortex, 22:1, hippocampus, 15:1, cerebellum, 15:1, midbrain, 9:1, and brain stem, 6:1. In spite of these regional differences in the population of cholinoreceptors associated with the AChE enzyme, there was no significant difference in the relative amount of the four isoenzymes in these regions. Since muscarinic cholinoreceptors predominate in the rat brain we also examined the isoenzyme pattern of AChE from electric eel, which contains purely nicotinic cholinoreceptors, and found the same isoenzyme pattern as that observed in each of the rat brain regions. Thus, association with different cholinoreceptor environments does not appear to influence the isoenzyme pattern of AChE. However, differences in the isoenzyme pattern were observed in rat brain AChE from different subcellular locations. Synaptosomal AChE was enriched in isoenzymes with high pI values when compared to microsomal AChE. This observation may explain why AChE isoenzymes with high pI values are inhibited in vivo more rapidly by soman than AChE isoenzymes with low pI values.

184.7 HALOTHANE ACTION ON ACETYLCHOLINE SYNETHESIS IN RAT BRAIN SYNAPTOSOMES, G.V.W. Johnson and C.R. Hartzell*. Research Department, The Alfred I. duPont Institute of the Nemours Foundation, Wilmington, DE 19899.

Cholinergic nerve terminals require choline and acetyl-CoA as precursors for acetylcholine synthesis. Choline is actively transported into the axonic terminals, while acetyl-CoA is produced directly from pyruvate by the action of pyruvate dehydrogenase (PDH) in the mitochondria. As a part of the overall study of neurotransmitter synthesis and the effects of inhalation anaesthetics, we report the effects of halothane on acetyl-CoA levels and choline acetyltransferase and PDH activities in the synaptosomes. Synaptosomes were isolated from rat cerebra, purified by centrifugation through a discontinuous Ficoll gradient and maintained in an isosmotic salt solution. Acetyl-CoA levels were measured using a radioisotopic technique. [^{14}C(U)]-oxaloacetate and citrate synthase were added to the sample and the amount of [^{14}C(U)]-citrate formed was determined (Pande & Caramancion, Anal. Biochem. 112:30, 1981). Initial concentrations of acetyl-CoA were found to be 19.5 ± 2.4 pmol/mg protein. During the first 10 minutes of incubation the acetyl-CoA levels in both control and 3% halothane-exposed synaptosomes dropped significantly to 4.5 ± 1.1 pmol/mg protein and 9.7 ± 1.7 pmol/mg protein, respectively. During the remaining 30 minutes of incubation synaptosomal acetyl-CoA levels remained elevated in the presence of 3% halothane when compared to untreated controls ($P<0.1$).

When measuring PDH activity, synaptosomes were pretreated with 0.2% digitonin to make the plasma membranes "leaky". Oxidative pyruvate metabolism was then measured using gas flow radiorespirometric techniques with (1-^{14}C)-pyruvic acid. Three percent halothane depressed PDH activity by 24%.

Choline acetyl-transferase (ChAT) was not significantly affected by halothane, nor did it change over the period of incubation. Control values for ChAT in the synaptosomes was determined to be 0.41 U/mg protein, while in the presence of 3% halothane synaptosomal ChAT activity was 0.43 U/mg protein.

Our interest is the action of halothane on acetylcholine synthesis. We demonstrated previously that halothane competitively inhibits choline uptake and depresses pyruvate metabolism. We have now shown that the depression of pyruvate metabolism is due in part to the decreased PDH activity. The observed increase of acetyl-CoA levels could be due to the decreased availability of choline. An alternate explanation includes the inhibition of NADH CoQ reductase by halothane. This effect would increase the reducing equivalents within the mitochondria thus inhibiting the citric acid cycle metabolism of acetyl-CoA. ChAT was unaffected by halothane, but this is not unexpected since it is probably a soluble enzyme.

185.1 DIETARY ASCORBIC ACID (AA): EFFECTS ON ETHANOL CONSUMPTION AND LIGAND BINDING IN GUINEA PIGS. Z.S. Dolinsky, S.L. Margoles* and E.G. Shaskan, Department of Psychiatry, University of Connecticut Health Center, Farmington, CT 06032.

Alcoholics have been shown to be deficient in AA. It is assumed that this deficiency is a result of poor nutrition concomitant with ethanol ingestion, but it is possible that low AA plays an etiological role in the development of alcoholism. Ethanol's effects are associated with changes in membrane fluidity. AA plays an important role in lipid peroxidation and has been demonstrated to attenuate the effects of ethanol.

The present study examined the influence of AA and a stressor upon ethanol consumption in adult male Hartley guinea pigs. Animals were given a two bottle choice of 10% ethanol or water, and fluid intake was measured daily for three weeks. After baseline measures, during which animals received AA supplemented chow, subjects were divided into two groups: A+ (Purina chow supplemented with 1g/kg AA) or A- (chow with no added AA). These two groups were further divided into a stress (2.5 min., 10°C. swim during the final nine days of fluid measurement) and non-stress group. Following the last day of testing, animals were sacrificed and AA levels were recorded in half brain and spleen. Since spiroperidol binding and MAO activity are membrane related phenomena, with AA and ethanol associations, these variables were also measured in corpus striatum and spleen.

The A- group was characterized by marked decreases in both spleen and brain AA relative to the A+ group. In addition, stress tended to raise the AA levels in the A+ group. There was an initial indication of an increase in ethanol consumption in the A- group relative to the A+ group: however, this increase was small and temporary. Stress in general, tended to decrease ethanol consumption. There was a positive correlation between ^{3}H-spiroperidol binding and MAO activity (tryptamine, 100μM) in the striatum of the A+ group. This was not seen in the A- group, or in spleens from either group.

Since AA deficiency only affected ethanol intake transiently, its role in mediating ethanol intake in guinea pigs is not clear. In this model system, however, the absence of a positive correlation between MAO activity and ^{3}H-spiroperidol binding in the A- group suggests that AA deficiency, secondary to ethanol intake, may alter membrane associated phenomena. This finding calls attention to the potential role which AA may play in mediating ethanol's influence on membrane function.

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185.2 EFFECTS OF ETHANOL ON RAT HYPOTHALAMIC LHRH RELEASE. A STUDY UTILIZING RIA AND IMMUNOCYTOCHEMISTRY. W.L. Dees*, N.H. McArthur, P.G. Harms*, and K.L. Farr*. Dept. of Veterinary Anatomy and Dept. of Animal Science, Texas A&M Univ., College Station, TX 77843.

To further understand the mechanism of action by which ethanol (ETOH) decreases plasma luteinizing hormone (LH) levels, the effects of multiple I.P. injections of ETOH (1.0-1.5 g/kg) or saline on hypothalamic luteinizing hormone releasing hormone (LHRH) and plasma LH were evaluated in intact and castrate male rats. After injections every 4 hours for 2 days or every 6 hours for 8 days, animals were killed and their tissues processed. Some animals were decapitated, brains removed, and blocks containing the hypothalamus (with median eminence, ME) were subjected to acetic acid extraction of LHRH, and the hormone quantitated via RIA. Brains from other groups of animals were removed following cardiac perfusion of 10% phosphate buffered formalin. Blocks containing the hypothalamus with ME were post fixed in Bouin's solution and processed for immunocytochemistry (ICC) using a specific antiserum to LHRH and the peroxidase-antiperoxidase technique. The RIA data showed that in all rats studied, hypothalamic LHRH was inversely correlated with plasma LH. In response to castration, both saline and ETOH treated rats showed a decrease in hypothalamic LHRH content with a concomitant increase in plasma LH; however, the ETOH treated animals retained significantly greater concentrations of LHRH, and showed significantly lower plasma LH levels when compared to saline treated controls. Likewise, ETOH treated intact animals showed significant increases in LHRH content, with LH levels remaining significantly lower than the saline treated intact controls. Values were considered significant if $P < 0.05$. Differences visualized immunocytochemically between saline or ETOH treated intact and castrated rats were consistent with the data which we obtained using RIA. Thus, these data using the complementary techniques of RIA and ICC support the hypothesis that ETOH diminishes or possibly inhibits hypothalamic LHRH release, and hence provides an explanation for depressed plasma LH levels observed in ETOH treated intact and castrated rats.

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185.3 INTRACELLULAR MEASUREMENT OF ETHANOL EFFECTS ON RAT LOCUS COERULEUS NEURONS IN A BRAIN SLICE PREPARATION. S.A. Shefner, T.H. Chiu* and E.G. Anderson. Depts. Pharmacol. and Physiol. Biophys., Univ. of Illinois College of Medicine, Chicago, IL 60680.

In vivo experiments using extracellular recording have shown that ethanol (ETOH) inhibits the firing of rat locus coeruleus (LC) neurons (Pohorecky, L.A. and Brick, J., Brain Res. 131:174, 1977). Intracellular recordings were made from rat LC neurons in a brain slice preparation during bath application of known concentrations of ETOH (10-60 mM), the concentration range found in brain during mild ETOH intoxication to sedation. Coronal slices (300μ) of rat pons containing the LC were cut on a vibratome and mounted totally submerged by artifical CSF in a small recording chamber (0.3 ml) kept at 37°C. Glass microelectrodes filled with 2 M KCl (60-100 MΩ) were used for recording.

Of 32 LC neurons (from 28 preparations) 44% were spontaneously active with firing frequencies ranging from 0.5 to 13 Hz. Bath application of ETOH (10-60 mM) inhibited the spontaneous firing in 7 of 8 neurons tested. This effect was dose-dependent both in terms of the extent of inhibition and the latency of the inhibitory effect. ETOH (10-60 mM) also caused changes in resting potential and input resistance (as measured by constant current hyperpolarizing pulses) in 15 of 16 cells. Three types of effects were noted: 1) membrane hyperpolarization (3-30 mV; n=5), 2) membrane depolarization (6-20 mV; n=5). (These effects were associated with a decrease or no change in input resistance.) 3) Biphasic responses (small transient initial depolarizations of 1-6 mV and variable changes in input resistance, followed by a prolonged hyperpolarization associated with a clear decrease in input resistance; n=5). In addition, ETOH (30 mM) was found to potentiate the effect of bath application of γ-aminobutyric acid (GABA; 0.5, 1 mM) on LC neurons (n=7). ETOH caused up to a 4-fold increase in GABA-induced depolarizations and up to a 2-fold increase in the GABA-induced reduction in input resistance.

It is possible that ETOH-induced inhibition of firing and changes in membrane potential and resistance may also be explained in terms of a GABA-mimetic effect or potentiation of tonic GABA-ergic input onto these cells. In fact, GABA (1 mM) completely inhibits spontaneous activity in LC cells (n=5). The variable membrane potential effects seen with ETOH are similar to the effects seen with GABA (n=21), in that, with KCl-filled recording electrodes GABA hyperpolarizes about 1/3 of LC neurons tested and depolarizes the remaining 2/3. This is presumably due to Cl^- leakage into the cell affecting the Cl^- equilibrium potential. GABA responses, however, are always associated with a decrease in input resistance whereas the initial transient depolarization seen with ETOH can be associated with an increase in input resistance. Secondly, GABA and ETOH applied to the same neuron do not always cause similar effects on membrane potential. These data suggest that ETOH effects on membrane potential and resistance can not be explained purely by a GABA-mimetic action, or potentiation of a tonic GABA-ergic input onto LC neurons. (Support: SAS, NIAA 7374; THC, NIAAA 2696).

185.4 CHANGES IN GAMMA-HYDROXYBUTYRIC ACID CONTENT IN THE LIVER AND BRAIN OF ETHANOL DEPENDENT RATS. F. Poldrugo* and O. C. Snead, Department of Pharmacology, Pediatrics and The Neuroscience Program, University of Alabama in Birmingham School of Medicine, Birmingham, Alabama 35233.

γ-hydroxybutyric acid (GHB) is a metabolite of γ-aminobutyric acid (GABA) and occurs naturally in brain. This compound has a number of diverse neurophysiologic and neuropharmacologic properties (Snead, Life Sci. 201, 1935, 1977). A marked increase of GHB in liver and brain has been reported to occur with single high dose administration of ethanol (Roth, Biochem. Pharmacol. 19:3013, 1970). However, there are no data concerning the effect of ethanol on liver and brain GHB. The object of these experiments was to examine the liver and brain content of GHB in an animal model of ethanol dependence.

Rats were allowed free access to a liquid diet containing either ethanol or isocaloric sucrose. After 1, 2, 4, 6, and 10 hours and in the morning of the first, second, fourth, sixth, and tenth day, the animals were decapitated and the GHB content of liver and brain determined by electron capture gas liquid chromatography. Blood ethanol was determined concomitantly by head space chromatography.

The GHB content of liver increased within the first hour of initiation of ethanol administration. This increase was significant (27.4 vs. 17.8 mM/gm. tissue; $p<.05$) in animals in which the blood ethanol level was greater than 60 mg/100 ml at the moment of sacrifice. At 8 a.m. of the first, second, fourth and tenth day of ethanol tolerance, the GHB liver content was still higher than control (17.9 vs. 5.7 mM/gm tissue $p<.01$).

While there were striking changes in the ethanol addicted animals in terms of liver concentration of GHB, there was very little change in concentration of GHB in brain in alcoholic rats. These data suggest that different mechanisms may be operative in GHB metabolism in the periphery vs. that in brain in ethanol dependent rats.

185.5 EFFECTS OF ACUTE ETHANOL TREATMENT ON RAT BRAIN OPIATE-PEPTIDE SYSTEMS. Scott N. Deyo, Joseph Rogers, William J. Shoemaker, Steven J. Henriksen, and Floyd E. Bloom. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute La Jolla, CA 92037.

Studies in our laboratory indicated a possible involvement of endogenous opioid systems in the acute effects of low doses of ethanol (ETOH). In a behavioral paradigm, naloxone reversed an ETOH-induced increase in the rate of punished responding (Koob et al., Subst.Alc.Act.Mis.1:447, 1980). ETOH also produces changes in hippocampal electrographic activity similar to that observed with low icv doses of β-endorphin (Henriksen et al., PNAS 75:5221, 1978). Subsequently, we have studied the neurochemical effects of ETOH on central opiate-peptide systems in the rat. An initial experiment demonstrated that ETOH induced a decrease in hypothalamic leu-enkephalin immunoreactivity (leu-enk-IR) two hours after administration. The effect on the leu-enk-IR was directly related to the blood ethanol concentration; however, there was no effect of ETOH on hypothalamic β-endorphin content. The following studies were, therefore, conducted with in vitro ETOH treatment (20, 40 and 100 mM) on the release of leu-enk-IR from rat globus pallidus slices. The lowest concentration (20mM) failed to produce a significant effect on leu-enk-IR release, but 40 mM ETOH produced a significant ($p<.025$; ∿ 30%) increase in K^+-stimulated, Ca^{+2}-dependent leu-enk-IR release. 100 mM ETOH produced an elevation of non-stimulated Ca^{+2}-dependent release of the peptide, and an inhibition of K^+-stimulated, Ca^{+2}-dependent leu-enk$_{IR}$ release. We observed a similar effect of the highest concentrations of ETOH on dopamine release from striatal synaptosomes. Interestingly, the effects of both concentrations of ETOH disappeared when ETOH was removed from the release buffers indicating these effects to be rapidly reversible.

Binding studies examining the effects of acute, in vitro ETOH on [^{3}H]-[D-Ala^2, D-Leu^5]-enkephalin (DADLE) saturation curves demonstrated no effect on the binding constants for DADLE in either the absence or presence of 10^{-4}M 5'-guanylyl imidodiphosphate, a stable GTP analog.

In conclusion our studies support the idea that ETOH in low to moderate (intoxicating) concentrations might act to increase the activity of enkephalin systems by increasing the rate of release of the peptide. (Supported by Grants NIDA 01785, NIAAA 03504 and NIAAA 07273.)

185.6 EFFECTS OF CHRONIC ETHANOL TREATMENT ON THE COUPLING OF CNS MUSCARINIC RECEPTORS AND PHOSPHOLIPID METABOLISM OF C57/BL MICE. Thomas L. Smith. Dept of Pharmacol., University of Arizona and Veterans Administration Medical Center., Tucson, Az. 85723

The disposition of CNS acetylcholine is known to be affected by both acute and chronic ethanol (E) administration. The purpose of the present work was to determine the effects of E treatment on CNS muscarinic receptors and their linkage to phospholipid metabolism. Adult male C57/BL6 mice were given a liquid diet containing 7% E (v/v) for eight days. This treatment has been previously demonstrated to produce functional tolerance and physical dependence. Chronic E treatment resulted in a significant increase (15%) in maximal specific binding of the muscarinic antagonist, ^{3}H-QNB, to isolated cholinergically enriched synaptosomes. No change in K_D was observed. These results are in agreement with a previous report (Life Sci 25:2173, 1979). In synaptosomes of untreated mice, muscarinic receptor activation by carbamylcholine enhances the in vitro incorporation of ^{32}P into phosphatidylinositol (PhI) and phosphatidic acid (PhA) by two fold. Chronic E treatment did not result in a corresponding increase in basal or carbamylcholine stimulated ^{32}P incorporation into these lipids. E given in vitro (300mM) reduced basal incorporation of ^{32}P into PhI and PhA of controls by c.a. 20%, while reducing carbamylcholine stimulated labeling of PhA by more than 30%. Synaptosomes from mice chronically treated with E were more resistant to the inhibitory effects of in vitro E on muscarinic enhanced PhA labeling. These results suggest that increased muscarinic receptor density after Chronic E treatment does not necessarily elicit a supersensitive muscarinic response. (supported by Veterans Administration Alcohol Research Grant).

185.7 ETHANOL AND K+ CONDUCTANCE: ENHANCEMENT OF THE AFTERHYPERPOLARIZATIONS (AHPs) FOLLOWING ACTION POTENTIALS OR DEPOLARIZATIONS EVOKED WITH AMINO ACIDS AND EFFECTS OF K+ AND Na+ CHANNEL BLOCKERS. P.L. Carlen, N. Gurevich*, E. Puil. Addiction Research Foundation Clinical Institute, Departments of Medicine, Physiology, Institute of Medical Science and Playfair Neuroscience Unit, Toronto Western Hospital, University of Toronto, and Departments of Anaesthesia and Pharmacology, Faculty of Medicine, University of British Columbia, Vancouver, B.C., Canada.

The AHPs following glutamate-evoked depolarizations of spinal and hippocampal neurons have been attributed to an electrogenic Na+-pump. A Ca^{2+}-activated K^+-conductance (Ca-gK) has been shown to be responsible for the slow AHPs which follow spikes in both types of neurons, and recently has been suggested to account for the AHP which follows the depolarization of hippocampal CA1 neurons with glutamate. We have attempted to resolve these conflicting explanations by examining the effects of low Ca-conditions (0 Ca^{2+} and 2.4 mM Mn^{2+}) and of ethanol (10mM), which increases Ca-gK following the post-spike AHP, probably by raising intracellular free Ca++. Ethanol also augments post-amino acid AHPs of hippocampal neurons in in vitro slices of guinea pig brain. The depolarizations elicited with aspartate or glutamate applied extracellularly by pressure ejection usually were not associated with large changes in membrane conductance (G_m); G_m tended to decrease with aspartate and increase with glutamate. Recovery from glutamate-depolarization was characterized by a conspicuous increase in G_m. Perfusion with low (Ca^{2+})-solution did not prevent the emergence of AHPs following such depolarizations in most cases, but completely blocked the post-spike slow AHPs and synaptic transmission. AHPs which followed aspartate- or glutamate-depolarizations were sometimes reduced by low (Ca^2) perfusion. Ethanol increased the amplitude and duration of AHPs following depolarizations evoked with the amino acids in either normal- or low-(Ca^{2+})-perfusion. These data suggest that (1) both a Ca-gK and an electrogenic Na-pump may be responsible for the AHPs following depolarizations evoked with aspartate or glutamate, and (2) ethanol increases both types of AHPs possibly through raising intracellular free Ca^{2+} thereby activating a gK. Indeed, the movement of Ca^{2+} into neurons may not be requisite for the activation of a K^+-conductance in the case of post-amino acid AHPs.

The following Na+ and K+ channel blockers were used in an attempt to block ethanol effects: perfused TTX, intracellularly injected QX222 (a local anaesthetic) and intracellularly injected cesium. Ethanol-induced increase of hyperpolarization, conductance increase and post-spike longlasting AHP all persisted.

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185.8 ALCOHOL PRODUCES ACUTE AND CHRONIC CHANGES IN NEURAL ALPHA-ADRENERGIC RECEPTORS. Michael E. Charness*, Adrienne S. Gordon and Ivan Diamond. Dept. of Neurology, Univ. of California, San Francisco, CA 94143.

Alcohol produces many effects on neural cell membranes. We have investigated the consequences of this interaction on neuroreceptors in NG108-15, a murine neuroblastoma x glioma hybrid cell line. Opiate (ORB) and alpha-adrenergic receptor binding (ARB) in whole cells was assayed by rapid filtration using ^{3}H-met-enkephalinamide and ^{3}H-Rauwolscine respectively. ETOH added acutely to cells decreased ARB by 40% in 100 mM and 90% in 600 mM ETOH. ORB decreased 20% in 100 mM and 70% in 600 mM ETOH. In order to search for tolerance to ETOH, cells were grown 4-6 days in the presence (chronic) or absence of 200 mM ETOH. ARB increased 2.6 + 0.6 fold in chronic ETOH cells compared to controls. ORB increased less consistently. Acute addition of ETOH to cells grown chronically in alcohol had less effect on ARB than in control cells. ORB sensitivity to ETOH was unaffected by chronic ETOH. Thus, while acute ETOH strikingly reduced ARB in NG108-15, chronic ETOH produced compensatory increases in ARB with tolerance to the acute effects of ETOH. This model system will be of value in studying the development of alcohol tolerance in neural cells.

185.9 MICROCOMPUTER-ASSISTED OPEN FIELD OBSERVATIONS OF ADULT MALE RATS TREATED WITH ETHANOL ON POSTNATAL DAYS 1-8. T.Sonderegger, S. Berney-Key*, J. Ritchie*, V. Tumblin*,J. Flowers*, and E. Zimmermann. Dept. of Psychol., U. Nebraska-Lincoln, Lincoln, NE. 68588:Dept. Anat., BRI, UCLA, Los Angeles,CA.90024

Few studies have examined effects of ethanol upon the postnatal rat although the central nervous system is still developing during this period. In the study reported here, known quantites of ethanol were administered directly to rat pups by intragastric intubation. Ethanol was in a 30% Sustagen (Mead Johnson) vehicle to counteract malnutrition effects. (This administration procedure has been shown to produce high levels of blood alcohol as long as four hours later.)

Eight litters of Charles Rivers CD albino rats were adjusted to litter sizes of 10 on Day 1 and pups randomly assigned to treatment groups: ethanol (E), Sustagen (S) or handled (H). Tapered doses, increasing to 4 g/kg on Days 4 and 5, were given twice daily to E pups; S pups received comparable volumes of isocaloric (sucrose) Sustagen and H pups were handled. On Day 4, E pups ceased to gain weight; one H pup of each sex from each litter was removed and placed with a nonlactating female until bodyweights matched littermate E animals. These animals were designated as Pair Underfed (PU) controls. Five other litters were used as unhandled (U) controls. Animals were weaned on Day 21 and housed in same-sexed pairs. Only data from male animals are reported.

Mortality was low (4/80). Both E and PU animals had lower body weights on Day 8 but otherwise group mean body weights did not differ (through Day 150). On Day 120, approximately, open field measures (5-min trials on 4 days during the dark cycle) were recorded directly on two microcomputers using programs for real time observations. These programs permitted timing of onset of events, summation of event times, frequency records of specific behaviors, records of episode sequences, and summation of episode times. Computer obtained activity data correlated well with similar data obtained by traditional methods.

E animals were less active (total squares entered) than other groups ($p<.05$) and exhibited longer start latencies ($p<.03$) than other groups. Activity levels of all groups decreased over days ($p<.01$). Various computer-timed and summated measures also discriminated among some groups. Computer-assisted recording procedures provide a finer focus on the subtle behavioral differences produced by treatment of the developing rat with relatively low doses of ethanol early in life and may prove valuable in assessments of effects of drug exposure in the developing organism.

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185.10 EFFECT OF MICROINJECTION OF ETHANOL ON TRIGEMINAL MOTONEURONS IN THE CHRONIC CAT. A. Baranyi* and M.H. Chase. Depts. of Physiology and Anatomy and the Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

Systemically injected ethanol has been shown to exert a variety of different neuronal effects which depend upon the dose administered and the response recorded. It depresses somatic reflexes of the spinal cord but facilitates spinal GABA-ergic and cholinergic synapses. Neither the influence of the animals' behavioral states nor that of anesthetic agents have been controlled for in these investigations; in addition, the specific responses that were examined have not been studied in isolation from the indirect effects elicited by the systemic route of administration. Accordingly, to attend to these problems we applied ethanol juxta- and intracellularly while recording from trigeminal motoneurons in chronic cats during the behavioral states of sleep and wakefulness. Five chronic cats were implanted with electrodes to monitor EMG, EEG and EOG activity and to stimulate the mesencephalic Vth nucleus (Mes V) and masseter nerve. Chronic intracellular paradigms (Chase et al., J. Neurophysiol., 44:349-358, 1980) were used to record 26 motoneurons during wakefulness, quiet sleep and active sleep. Ethanol (50-150 picoliters of 0.5-2.5M solutions) was injected into and next to the recorded cell by pressure via a combined multibarrel electrode. Extracellular ethanol injection in 17 neurons induced the following qualitatively different dose-dependent responses. (1) Small doses of 0.5-1M ethanol induced a reversible decrease in firing activity (for 1-2 minutes) and a 10-30% reduction in the orthodromic and antidromic spike amplitude. The peak latency of Mes V EPSPs increased, while their amplitudes and decay time were attenuated. No changes in membrane potential or resistance were observed. (2) Increased doses (1-2M) caused, in addition, membrane hyperpolarization of 3-8 mV preceded by a short depolarizing phase. The membrane resistance decreased during hyperpolarization and the synaptic and action potentials were suppressed. (3) Large doses of ethanol (2.5M) induced a transient excitation which ended in a depolarizing block of neuronal activity. Intracellular injection of 2.5M ethanol in 9 neurons caused a reversible (for 0.5-1 minute) depolarization together with an attenuation in the spike and EPSP amplitude. The cellular effect of microinjected ethanol on trigeminal motoneurons was found to be state-independent. We conclude that ethanol induces a relatively specific inhibition of Na-activation which was characteristic of all doses and which was revealed with both juxta- and intracellular routes of injection. On the other hand, the hyperpolarizing action of ethanol is likely due to different mechanisms, possibly those involving changes in membrane receptor activity.

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185.11 ETHANOL INCREASES THE FIRING RATE OF DOPAMINERGIC NEURONS G.P. Mereu* and G.L. Gessa*, Institute of Pharmacology, University of Cagliari, 09100 Cagliari, Italy.

The effect of the acute administration of ethanol on the electrical activity of dopaminergic neurons in the substantia nigra, pars compacta (SN-DA neurons) was studied in unanesthetized paralyzed rats using the microelectrode single unit recording technique. Ethanol, injected via femoral vein in doses from 0.1 to 8.0 g/kg, produced a biphasic effect. In doses ranging from 0.5 to 2.0 g/kg, it produced a dose-related increase (from 25 to 90%) in the firing rate, an effect which began approximately 30 to 60 sec after treatment and persisted for 10 to 120 min (in proportion to the dose). During ethanol stimulation, the firing pattern was characterized by a progressive increase in long "trains" discharge with decreased amplitude and increased duration of the action potential. On the contrary, high doses of ethanol (over 3.5 g/kg) inhibited the dopaminergic activity until the complete suppression of cell discharge at doses of about 6.0 g/kg. Gradual recovery from inhibition began 5 to 30 min after treatment in proportion to the ethanol dose. The stimulant effect of ethanol was readily reversed by the subsequent injection of apomorphine (20 ug/kg), while the inhibitory response to high doses was not antagonized by haloperidol in doses up to 1.0 mg/kg. Neither response was modified by intravenous naloxone up to a dose of 5 mg/kg. Our findings support the hypothesis that the alterations of dopaminergic activity might play an important role in ethanol intoxication.

186.1 PARTIAL PURIFICATION AND CHARACTERIZATION OF DOPAMINE RECEPTORS FROM RAT STRIATUM. J.Y. Lew* and M. Goldstein. Department of Psychiatry, New York Univ. Med. Center, New York, N.Y. 10016.

The present study was undertaken to purify and to characterize soluble dopamine (DA) receptors from rat striatal membranes. The DA receptors were solubilized by using the detergent CHAPS (Lew, J.Y. and Goldstein, M., Eur. J. Pharmacol., 72:403, 1981). The membrane-bound DA receptor was solubilized with 10 mM CHAPS and subsequently centrifuged at 100,000 x g. The supernatant was applied to a lectin wheat germ (WGA) agarose column (0.8x8.0 cm). The unbound proteins were removed by washing the column with 20 ml of Tris HCl buffer, pH 7.0 containing 2 mM CHAPS. The receptor was eluted with 20 ml of 0.1 M N-acetylglucosamine. The binding of ^{3}H-spiroperidol (^{3}H-Spi) to the partially purified solubilized DA receptor is displaced by nanomolar concentrations of (+) butaclamol and haloperidol, but not by (-) butaclamol. In some experiments, the solubilized DA receptor was pre-labelled with ^{3}H-Spi and subsequently adsorbed on WGA-agarose column. The ^{3}H-Spi prelabelled receptor was eluted from the column with N-acetylglucosamine. The results of this study indicate that the striatal DA receptor is a glycoprotein which probably contains N-acetylglucosamine. The adsorption of the ^{3}H-Spi labelled receptor on WGA-agarose column suggests that neuroleptics bind to a different site of the receptor than the lectin. Studies are now in progress to further characterize the partially purified DA receptor. Supported by NINDS 06801 and NIMH 02717.

186.2 SULPIRIDE INHIBITS DA-STIMULATED ADENYLATE CYCLASE ACTIVITY IN STRIATUM FROM SEXUALLY IMMATURE AND CASTRATED MALE RATS. M. Gnegy, G. Treisman* and A. Bernabei*, Dept. Pharmacology, Univ. Mich. Med. Sch., Ann Arbor, MI 48109.

Sulpiride (SPD), a substituted benzamide antipsychotic drug, is considered a selective antagonist at dopamine (DA) D-2 receptors because it does not inhibit DA-stimulated adenylate cyclase (DA-AC) activity. We found that SPD can block DA-AC activity in rat striatum from sexually immature (S-IMM) or adult male castrated (CAS-M) rats. Chronic treatment with SPD (20 mg/kg i.p. 2 x's daily, 15 d) resulted in supersensitivity of DA-AC activity in Ka (K_a^{app}) for DA was decreased 4-fold in the SPD-treated rats while the apparent Vmax (V_{max}^{app}) remained unchanged. There was no change in DA-AC after chronic SPD in adult or SHAM groups. Kinetic constants for DA-AC in SHAM, S-IMM and CAS-M groups are given below (Ka in μM; Vmax in pmol/min/mg protein):

	VEHICLE[a]		SPD	
	K_a^{app}	V_{max}^{app}	K_a^{app}	V_{max}^{app}
SHAM	1.4 ± 0.4	111 ± 12	1.6 ± 0.3	86 ± 11
S-IMM	4.7 ± 1	152 ± 20	1.1 ± 0.2*	110 ± 9
CAS-M	3.6 ± 0.8	137 ± 30	1.0 ± 0.3*	104 ± 21

[a] N = 5 for all groups; value is ± S.E.M. *P<0.02 as compared to VEHICLE control.

Basal and GTP-stimulated AC was not changed in any group after chronic SPD.

In in vitro studies we found that SPD at nM levels could inhibit the ability of low concentrations of DA to stimulate AC activity in striatal particulate fractions from S-IMM, CAS-M or adult ovariectomized female (CAS-F) rats. SPD did not affect basal, GTP or stimulation in S-IMM and CAS-M striatum, as did haloperidol and fluphenazine. The Ki's for SPD were very close to the reported inhibiting DA-AC from S-IMM, CAS-M and CAS-F rat striatum are 15 nM, 21 nM and 8 nM, respectively. The inhibition by SPD may be general for D-2 drugs because metoclopramide also inhibited DA-AC in striatum from CAS-M and CAS-F rats but not SHAM controls. These results suggest that sex hormones play a role in determining the pharmacological profile of DA receptors in rat striatum. The effects seem to be activational since they can be blocked by castrating adult rats. Supported by MH 36044-01.

186.3 SOLUBILIZATION OF A GUANINE NUCLEOTIDE SENSITIVE FORM OF THE D-2 DOPAMINE RECEPTOR FROM BRAIN REQUIRES AGONIST OCCUPANCY. Tan Creese and S.E. Leff. Dept. of Neurosciences, Univ. of California, San Diego, Sch. of Medicine, La Jolla, CA 92093.

The binding of the dopaminergic antagonist ^{3}H-spiperone and the agonist ^{3}H-dopamine has been partially characterized in both intact canine caudate membranes and digitonin-solubilized extracts. Soluble ^{3}H-spiperone binding sites (K_d~1nM) demonstrate a drug specificity comparable to the D-2 dopamine receptor previously characterized in rat and calf striatal membranes. Agonist competition for ^{3}H-spiprone binding in membranes shows that dopamine is potent (IC_{50}~3μM) and the curve is shallow (n_H 0.5). Furthermore, dopaminergic agonists' affinities are decreased in the presence of 0.1mM GTP. However, agonist competition for ^{3}H-spiperone binding in solubilized preparations is not GTP sensitive and reveals only low affinity binding characteristics (IC_{50} 10μM), n_H 1.0 for dopamine). Detergent treatment of caudate membranes after prior incubation with dopamine releases binding sites whose agonist affinity, determined by ^{3}H-spiperone competition, is guanine nucleotide sensitive. Additionally, specific high affinity ^{3}H-dopamine binding in the solubilized preparation is demonstrated only when membranes are prelabeled with ^{3}H-dopamine. GTP increases the dissociation rate of ^{3}H-dopamine from such prelabeled binding sites. These results indicate that the preservation of a guanine nucleotide sensitive form of the D-2 receptor after digitonin solubilization is dependent upon the presence of an agonist-receptor (A R) complex at the time of solubilization. Supported by PHS MH32990.

186.4 EFFECTS OF DURATION AND RATE OF STIMULATION ON THE NEGATIVE FEEDBACK REGULATION OF DOPAMINE RELEASE. L.X. Cubeddu* and I.S. Hoffmann*. (SPON: W.E. Stumpf). Div. Clin. Pharmacol., Univ. of North Carolina, Chapel Hill, N.C. 27514. Dept. Pharmacol., UCV, Apdo N. Granada, 40109, Caracas, Venezuela.

The present study was designed to study the operational characteristics of the feedback loop for regulation of dopamine (DA) secretion, in striatal dopaminergic neurons. Rabbit neostriatal tissue slices were incubated with ^{3}H-DA (0.3μM, 30 min) and then superfused at 1ml/min with physiological solution (pH 7.4, 37°C). Slices were stimulated twice (S_1 and S_2) with electrical pulses (12V, 1ms) at 60 and 125 min of perfusion. Drugs were added 15 min prior to S_2 and continued throughout the experiment. The increase in ^{3}H-overflow evoked by stimulation was expressed as % of tissue ^{3}H. Transmitter overflow was totally inhibited by reducing by 1/10 the Ca^{++} concentration of the perfusion fluid. Although transmitter overflow showed an increase with greater number of pulses (30-360 pulses), flat frequency-release curves (0.1-10Hz) were obtained for the striatal dopaminergic neurons. Haloperidol (0.03-0.3μM) and sulpiride (1μM) enhanced ^{3}H-overflow without affecting its metabolism or time course. Maximal enhancement of release by both neuroleptics was seen with short trains (30-60 pulses) and high frequencies of stimulation (3-10Hz). These drugs had negligible effects at lower rates (0.1-1Hz) or longer trains of stimulation (360 pulses). Therefore, the slope of the frequency-release curve was markedly increased by DA antagonists ($P<0.001$). Apomorphine inhibited transmitter overflow in a concentration-dependent manner (0.01-1μM). However, the inhibitory effect of apomorphine was markedly reduced at high rates of stimulation (10Hz). Haloperidol and sulpiride antagonized the inhibitory effects of apomorphine on transmitter release. These results indicate that activation of presynaptic DA-receptors, and thus facilitation of release by DA-antagonists is highly dependent on the rate and duration of stimulation of striatal dopaminergic terminals. In these neurons the feedback loop seems to act physiologically to depress the slope of the frequency-release curve.

186.5 PRESYNAPTIC REGULATION OF ENDOGENOUS DOPAMINE RELEASE FROM RAT STRIATAL SYNAPTOSOMES. Jiang, D.-H., Wagner, H.R., Yablonskaya-Alter*, E., Reches, A., Fahn, S., Department of Neurology, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

Brain synaptosomes release neurotransmitters under depolarizing conditions. We have used high pressure liquid chromatography (HPLC) and electrochemical detectors (EC) to measure the release of endogenous dopamine (DA) from rat striatal synaptosomes. A crude synaptosomal pellet (P2) was prepared by established methods. The final pellet was resuspended in oxygenated Krebs buffer containing 10^{-4}M pargyline and preincubated at 37°C/10 min. Additional buffers were added to a final volume of 200 ul (protein conc.=1 mg/ml) and the incubation was continued an additional 10 min unless otherwise specified. The incubation was stopped by centrifugation and the supernatant was assayed for DA using HPLC with EC detection. Calcium (Ca^{++})-dependent DA release was concentration-dependent in the presence of increasing concentrations of K^+. Release was constant above 55 mM K^+ with a maximum release level of 181.0 pmols DA/mg protein. Half-maximal DA release occurred at 25 mM K^+. Release in the presence of 25 mM K^+ was biphasic with an early rapid phase (<30 sec) and a slower and larger second phase. Equilibrium conditions occurred within 10 min and remained stable for at least 10 additional minutes. Release in the presence of 25 mM K^+ was enhanced by the dopamine antagonist, haloperidol (10^{-5}M). Apomorphine, a dopamine agonist, partially (35%) inhibited release at high concentrations (10^{-5}M). Pergolide, an ergot derivative with DA agonist properties, did not affect release. This system appears to provide an in vitro means of studying the presynaptic regulation of striatal DA release.

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186.6 EFFECTS OF KETANSERIN (R 41 468) AND PIRENPERONE (R 47 465), TWO NOVEL ANTAGONISTS OF THE $5HT_2$ SEROTONERGIC RECEPTOR, ON PROLACTIN SECRETION IN MALE RATS. M. Simonovic, G. A. Gudelsky and H.Y. Meltzer. Dept. of Psychiatry, University of Chicago Pritzker School of Medicine, Chicago, IL. 60637.

In vitro binding studies have demonstrated that ketanserin (KET) and pirenperone (PIR) possess high affinity for the serotonin ($5HT)_2$ receptor (Ki = 2.1 nM for both compounds) and no appreciable affinity for the $5HT_1$ receptor. The affinity of PIR for the dopamine (DA) receptor is relatively high (Ki = 15.8 nM) compared to that of KET (Ki = 220 nM). Biochemical and behavioral studies suggest that these two compounds are selective $5HT_2$ antagonists.

Rat prolactin (PRL) secretion is under tonic dopaminergic inhibition mediated by pituitary DA receptors. Hence, drugs known to interfere with dopaminergic transmission cause increased release of this hormone. Rat PRL secretion can also be stimulated by 5HT or 5HT agonists acting on central, probably hypothalamic, 5HT receptors. The pharmacology of these receptors is still obscure.

KET (1 - 10 mg/kg, i.p.) had no effect on resting serum PRL levels in male rats. In addition, KET (0.5 - 5 mg/kg, ip) did not block the stimulation of PRL secretion by the 5HT precursor, 5-hydroxytryptophan (100 mg/kg, ip) or by the 5HT agonist, 5-methoxy-N,N-dimethyltryptamine (5 mg/kg, ip). In contrast, PIR (0.001 - 10 mg/kg, ip) produced a rapid and sustained rise in serum PRL levels. The effect of PIR on PRL secretion was dose-related between 0.001 and 0.1 mg/kg (half-maximal effect was produced by 0.01 mg/kg); doses greater than 0.1 mg/kg had no further effect on serum PRL levels. The PRL-releasing effect of PIR (0.1 mg/kg, ip) was completely antagonized by two DA agonists, apomorphine (0.25 mg/kg, sc) and d-LSD (0.05 mg/kg, ip), while the pretreatment with 5HT antagonists, cinanserin (10 mg/kg, ip), cyptoheptadine (5 mg/kg, ip) KET (5 mg/kg, ip) or pizotifen (5 mg/kg, ip) had no effect. In addition, PIR (10^{-6} M) completely blocked the inhibitory effect of DA (10^{-6} M) on PRL release by pituitary glands in vitro.

The inability of KET to block serotonergic stimulation of PRL release does not rule out a $5HT_2$-mediated mechanism, as this compound is excluded by the blood-brain barrier. Its inability either to stimulate or to inhibit PRL secretion in vivo suggests that the interaction of KET with pituitary DA receptors is negligible. On the other hand, the stimulation of PRL secretion by PIR is probably due to its ability to block pituitary DA receptors. This conclusion is consistent with the high affinity of PIR for DA receptors and is further supported by our finding that it blocks the effect of DA on PRL secretion in vitro. However, a possible interaction of PIR with alpha adrenergic and histaminergic receptors should also be considered. (Supported, in part, by USPHS MH 30039).

186.7 **EFFECTS OF NA-ASCORBATE ON ^{3}H-ADTN BINDING.**
P. Hartig*, P. Sheth*, S. Finch* and D. Black*
(Spon: M. Larrabee), Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

Conflicting reports have appeared on the effect of ascorbate on the binding of DA agonists. We have confirmed that low (μM) levels of ascorbate cause large increases in the total binding of ^{3}H-ADTN to bovine caudate or rat striatal membranes prepared and assayed by the method of Kaayulp and Neff (Life Sciences 26, 1837 (80)). In contrast, ^{3}H-ADTN binding is not increased at low ascorbate concentrations when a P2 membrane preparation from bovine or rat brain is used. TLC analysis shows that high levels (mM) of ascorbate protect ADTN from oxidation during the binding assay but low levels (μM) do not. In the presence of low levels of ascorbate, the membrane preparations provide varying degrees of partial protection against oxidation. Using the Kaayulp and Neff membrane sample, ^{3}H-ADTN binding at high ascorbate levels shows the properties expected for dopamine receptor sites while at low ascorbate levels, dopamine agonists show reduced affinities for ^{3}H-ADTN binding sites and dopamine antagonists are very weak competitors.

These results show that different membrane preparation and assay methods play an important role in the effect of ascorbate on ^{3}H-ADTN binding. The enhanced ^{3}H-ADTN binding seen at low ascorbate levels in certain preparations is correlated with an increased oxidation of ADTN under these conditions. Dopamine agonists and antagonists display reduced affinities for this enhanced ^{3}H-ADTN binding.

(Supported by USPHS grant MH 36877 and NSF grant BNS 8108080)

186.8 ESTROGEN SENSITIVE DOPAMINE RECEPTORS IN RAT BRAIN: LOCALIZATION AND DISTRIBUTION. Robert E. Hruska and Karen T. Pitman*. Dept. Biochemical Pharmacology, School of Pharmacy, SUNY, Buffalo, NY and Neurotoxicology Section, NINCDS, NIH, Bethesda, MD.

Estrogen may have two separate and distinct effects. The first is a rapid effect and is measured as a decrease in dopamine (DA) function. This could be mediated through other neurotransmitter systems, but does not appear to involve the DA receptors labelled by ^{3}H-spiperone. The second effect develops slowly, may require pharmacological doses of estrogen, may require prolactin, and is measured as an increase in striatal DA receptor density. Our experiments only address the second effect of estrogen.

We have shown previously that estrogen treatment increases the density of striatal DA receptors. We have now localized this response and isolated it to one population of striatal neurons. Adult, male rats were injected with 17β-estradiol valerate in sesame oil(125 μg/rat, s.c., 6 days prior). The DA receptors labelled by ^{3}H-spiperone were measured in striatum, N. accumbens, cortex, hippocampus, and pituitary. Only the DA receptors in the striatum were increased in density after estrogen treatment. Those in the N. accumbens were not altered. In the cortex two sites were labelled and neither the DA site or the second site were changed by estrogen treatment. The DA receptors in the hippocampus were decreased in density. The number of DA receptors in the whole pituitary was not altered, but the pituitary was increased in weight and protein content. Expression of the results on a wet weight or protein basis made it appear that there was a decrease in density. Since the pituitary is still hypertrophied 6 days after estrogen treatment, we propose that the increase in striatal DA receptor density is the result of continuous exposure to estrogen. This argues against a withdrawal phenomena.

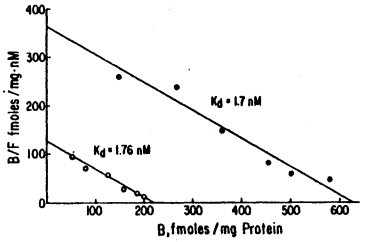
The striatal DA receptors, increased in density by estrogen treatment, belong to one population. Striatal injection of kainic acid destroys the neuronal cells originating in the striatum, but does not affect those originating outside the striatum. The unilateral injection of kainic acid decreased the density of the striatal DA receptors by about 30%. While estrogen treatment increased the density of the striatal DA receptors in the intact striatum by about 20%, it did not alter the density in the kainic acid lesioned striatum (i.e. the density remained decreased by 30%). The estrogen sensitive DA receptors are located on neurons originating in the striatum. This localization of DA receptors is similar to that found after haloperidol treatment and further supports our hypothesis that at least part of the effects of estrogen and haloperidol may be mediated similarly through an increase in prolactin levels. These results further demonstrate the specific response of striatal DA receptors to estrogen treatment. (Supported in part by RDF from Res. Fdn. of SUNY).

186.9 PREINCUBATION WITH DOPAMINE REVERSES THE DECREASE IN D-3 BINDING INDUCED BY 6-HYDROXYDOPAMINE OR RESERPINE BUT NOT BY KAINIC ACID. B.I. Schweitzer * and N. G. Bacopoulos. Departments of Pharmacology and Psychiatry, Dartmouth Medical School, Hanover, NH 03755.

The number of stereospecific binding sites (Bmax) of 3H-dopamine (3H-DA) and 3H-apomorphine (3H-APO) was reduced by 50-60% 4 or 20 hr after reserpine (4.0 mg/kg im) or six days after the intrastriatal injection of 30 ug 6-hydroxydopamine (6-OHDA). Both treatments lowered endogenous DA by 95% or more. In addition to lowering the Bmax, the depleting agents also lowered the equilibrium dissociation constant (Kd) to 47% (3H-DA) or 65% (3H-APO) of control. Preincubation of homogenates of depleted or lesioned striata with added DA at 37 degrees C for 30 min, restored the binding parameters of 3H-DA to control values and reversed the change in the Bmax of 3H-APO. The ED50 of this effect of DA was 50 nM, and it was mimicked by catecholamines but not by serotonin.

The intrastriatal injection of kainic acid (2.5 ug) also lowered the Bmax of 3H-agonists by 60-70% without changing the Kd or DA content. Preincubation of homogenates of kainic acid-lesioned striata (endogenous DA = 355 nM) did not reverse the decrease in Bmax. We conclude that the changes in D-3 receptors induced by 6-OHDA are due to a reversible change in receptor conformation rather than a permanent loss. In contrast the destruction of intrinsic striatal neurons by kainic acid produced an irreversible loss of receptors. These results explain the discrepancy between previous reports (Nature 274:278, 1978; European J. Pharmacol. 56: 277, 1979) and suggest that dopaminergic 3H-agonist receptors are located on neurons postsynaptic to the nigrostriatal projection. The concept that DA stimulates stereospecific 3H-agonist binding to subsequently washed membranes of rat brain, which we proposed (Life Sciences, 29:2407, 1981) is supported by evidence recently reported by another laboratory (Life Sciences, 30:1587, 1982). Supported by research grant MH33958.

FIGURE 1



Rats were sacrificed 6 days after kainic acid lesion and 3H-DA binding was measured as described previously (Life Sciences, 29: 2407, 1981). Empty circles lesioned striatum; filled circles: contralateral control side. Kd is the slope of the Scatchard plot.

186.10 PROPERTIES OF D-2 RECEPTORS LABELLED BY (^{3}H)BUTYROPHENONES IN HOMOGENATES OF RAT BRAIN. P.M. Beart*, D.J. de Vries*, H.W. Rzeczniczak* and A.L. Gundlach* (SPON: I. Darian-Smith). University of Melbourne, Clinical Pharmacology and Therapeutics Unit, Austin Hospital, Heidelberg, Victoria 3084, Australia.

Although (^{3}H)spiperone is a ligand for neuroleptic receptors, a major problem in accepting (^{3}H)spiperone binding as an association to dopamine receptors has been the low potency of dopaminergic agonists in displacing the binding. Recent evidence suggests that (^{3}H)spiperone binds to multiple sites (Beart, Trends Pharmacol. Sci. 3, 100-102, 1982), and this study investigated the properties of these binding sites in dopaminergic regions of rat brain.

Sprague-Dawley rats (150-300 g) were used and the corpus striatum, nucleus accumbens septi (NAS) and tissue enriched in the ventral tegmental area (VTA) were dissected from freshly removed brains. Membranes were prepared by homogenisation, centrifugation and washing, and used for binding studies. Specific (^{3}H)spiperone binding was stereospecific, rapid, saturable, reversible and showed no cooperativity in all regions studied. Saturation data (non-specific binding defined with 1 µM spiperone or 10 µM cis-flupenthixol) were more consistent with a two site model with the binding consisting of high and low affinity components, although in VTA resolution was difficult due to the low density of binding sites. In striatum and NAS the apparent K_D values for the high affinity sites were 93 ± 32 pM and 74 ± 30 pM respectively, while those for the low affinity sites were 4.0 ± 0.5 nM and 4.1 ± 0.8 nM respectively. Sites of high affinity represented 25, 15 and 5% of binding in the striatum, NAS and VTA respectively. The results of dissociation experiments in all three regions were also better described by a two component model.

The high affinity sites had properties consistent with a D-2 receptor. Drug displacement studies employing 25-50 pM (^{3}H)spiperone yielded K_i values for dopaminergic antagonists of 0.1-80 nM, while agonists had K_i values in the range 5-200 nM. Ergots and sulpiride were potent displacers of binding, while SKF 38393 (D-1 agonist) had a K_i of 13 µM. (^{3}H)Domperidone gave similar results, but bound less tightly to the high affinity site (K_D 0.2 nM).

Guanine nucleotides (100 µM) modulated (^{3}H)spiperone binding and produced 2-fold decreases in the affinity of apomorphine, ADTN, pergolide and lergotrile for the high affinity (D-2, defined with 5 µM (±)sulpiride) site. Hill coefficients were unchanged, as were the K_D and B_{max} values. GTP and guanylylimidodiphosphate failed to alter the interaction of haloperidol, sulpiride and bromocryptine. Manganese (1 mM) also did not modify the actions of the latter three drugs, whereas the affinity of the agonists was increased. Supported by National Health & Medical Research Council , Australia.

186.11 IN VITRO DOPAMINE RECEPTOR MODIFICATION BY AMANTADINE. R.M. Allen. Psychiatric Clinical Research Laboratory, Veterans Administration Medical Center, Dallas, Texas 75216.

Amantadine has been shown to prevent neuroleptic induced DA receptor hypersensitivity (Allen et al, Eur. J. Pharmacology, 65:313-315, 1980) and to have direct effects on both agonist and antagonist ligand binding (Allen, Soc. for Neurosci. Abstr., 1981). Bacapoulus (Life Science, 29:2407-2414) has demonstrated in vitro DA binding site plasticity with ^{3}HDA but not ^{3}H spiperone binding. In this study fresh dog striata were homongenized in 100 vol. Tris salt with .01% ascorbate and 1mM EDTA buffer, pH 7.4, preincubated for 10 min at 37° C, washed X 2 at 20,000g and resuspended in 50 volumes buffer. Inhibitions curve using 200pM spiperone as the ligand and apomorphine in concentrations ranging from 5nM to 10 µM were performed. The assays were performed with the above buffer, incubated at 22° C for 20 minutes, and rapidly filtered and washed 3 times with Tris Hcl pH 7.0, and counted on a Searle Mark II liquid scintillation counter. Apo above produced 3 definite inhibition points at 125nM, 235nM and 2500nM. The addition of 5nM amantadine abolished the very high and low affinity points and yielded an IC_{50} value [defined by 10^{-5} (+) Butaclamol] of 260nM. The finding of an intermediate but relatively high affinity site would suggest the presence of a D_4-like site with similar affinities for both agonist and antagonist ligands. Amantadine appears to modify DA receptor sites to a D_4-like configuration. Implications of this finding in the clinical activity of amantadine as well as alternative interpretations of the data will be discussed.

186.12 FOREBRAIN DOPAMINE RECEPTORS ARE LATERALIZED AND MODULATED BY ELECTRICAL SELF-STIMULATION OF VENTRAL TEGMENTUM IN RAT. Linda H. Schneider, Edgar E. Coons* and Randall B. Murphy, Departments of Psychology and Chemistry, New York University, New York 10003.

In rats, each implanted with an electrode in the left-side A10 cell region, we used [^{3}H]-spiroperidol (0.6nM) binding to examine and compare the effects of electrical stimulation on dopamine receptor levels in the ipsilateral and contralateral A10 terminal zones (specifically, olfactory tubercle assayed together with nucleus accumbens = OT/NA). As a within-subject control, we also examined dopamine receptor binding in the ipsilateral and contralateral striatum. Three different groups of rats were employed: unimplanted controls (N = 12), implanted but unstimulated controls (N = 19), and stimulated animals (N = 22). Binding was examined using standard filter binding techniques applied to individual sides of the individual rats' dopamine terminal zones.

We observed the unexpected result that in unimplanted animals levels of binding were significantly ($p < .01$) greater in left than in right striatum. An opposite (right > left) hemispheric difference in binding was observed in OT/NA but failed to reach significance. In the implanted but unstimulated rats a significant ($p < .002$) difference in the same direction as in the unimplanted rats' OT/NA data was evident; however, the striatal (left > right) hemispheric differences observed in the unimplanted controls were now reduced below significance. In the stimulated rats (which were sacrificed 90 minutes after the second of two electrical self-stimulation sessions) a reversal from control patterns in OT/NA dopamine receptor binding was observed and was found to be highly significant ($p < .002$) with respect to the implanted but unstimulated rats. Neither implantation nor stimulation resulted in significant side-to-side differences in striatal binding in contrast to differences seen in the unimplanted control group. We conclude that the binding effects observed after electrical stimulation arise as a direct result of this treatment because the left-right pattern of OT/NA dopamine binding was reversed in a separate study in which the electrical stimulation was delivered to the right A10 region instead of the left.

These data suggest that in studies of the ascending dopamine systems it is insufficient to utilize as a control the dopamine terminal zones contralateral to a treatment. The data also support the concept that mechanisms of interhemispheric receptor regulation exist in the rat.

186.13 AFFINITY CHROMATOGRAPHY OF THE NEUROLEPTIC DOPAMINE RECEPTOR. L. Antonian*, D.I. Schuster and R.B. Murphy. Dept. of Chemistry, New York University, New York, N.Y. 10003.

An affinity chromatography matrix has been devolped for extensive purification of the canine dopamine receptor. A haloperidol derivative, haloperidol hemisuccinate, has been specially synthesized as the ligand for attachment to the affinity support via a hydrophilic spacer arm. Experiments were performed on the affinity gel as well as on control gels. Batch experiments with a control gel showed 35-69% adsorption of the neuroleptic receptor, whereas batch experiments with the haloperidol affinity gel demonstrated adsorption of 85-100% of the solubilized dopamine receptor. The receptors are assayed by specific binding of [^{3}H]spiroperidol.

Chromatography of the solubilized dopamine receptor on the control gel resulted in the elution of two distinct protein fractions. Only one of the protein fractions showed specific binding of [^{3}H] spiroperidol which also represented a 2-4 fold purification of the neuroleptic receptor. Purification of the neuroleptic/dopamine receptor on the haloperidol affinity gel also yielded a protein profile similar to that of the control gel, yet the neuroleptic receptor remained adsorbed on the column. Conditions for specific elution of the neuroleptic receptor from the affinity support are under investigation.

186.14 DOPAMINE RECEPTORS ON HUMAN PARATHYROID GLANDS AND PARATHORMONE SECRETION AFTER DOPAMINERGIC DRUGS. I.N. Ferrier*, C.A. Bloxham* and A.J. Cross. Division of Psychiatry, Clinical Research Centre, Harrow, Middlesex, U.K., HA1 3UJ.

Dopamine (DA) receptors have been described on bovine parathyroid glands and, in view of their mediation of DA stimulated adenylate cyclase, are held to be D1 receptors. It has been shown that DA stimulates parathormone(PTH) secretion in cattle both in vitro and in vivo. The present study was undertaken in an attempt to determine if D1 receptors are present in human parathyroids and if PTH secretion responded to DAergic drugs.

Parathyroid glands were obtained at autopsy and were stored at -40 °C. Ligand binding assays contained crude membrane fractions and were incubated at 37 °C with 0.25 nM ^{3}H-piflutixol (^{3}H-PF). Specific binding was defined as that displaced by 10 µM (+) butaclamol. Saturation analysis revealed that the specific binding of ^{3}H-PF was saturable and was consistent with a single class of binding site (K_D = 0.32 nM, Bmax = 240 fmol/mg protein). By contrast no displaceable ^{3}H-spiperone binding was evident. The order of drug potency in inhibiting ^{3}H-PF binding to human parathyroids (IC_{50} domperidone >> DA > bromocriptine >> apomorphine (APO) >> haloperidol >> cis-flupenthixol (FLUP)) is similar to that in human brain.

PTH and prolactin (PRL) were measured by RIA (PTH antiserum C-terminal directed (residues 34-84)) in serum samples from 15 normal controls before and at 15 minute intervals after the administration of APO (0.75 mg s.c.), FLUP (3 mg p.o) or metaclopramide (MCP: 10 mg i.v.) and from 7 acute schizophrenic patients before and at weekly intervals after FLUP (3-8 mg/day p.o.). Rapid and sustained elevations of PRL after FLUP and MCP and suppression after APO, but no significant changes in PTH after any drug regimen, were observed.

These results suggest that while D1 binding sites are present on human parathyroids, the measurement of PTH after the administration of DAergic drugs is unlikely to provide a test of D1 receptor function in man.

187.1 AGONIST-SPECIFIC CHANGES IN THE PROPERTIES OF β-ADRENERGIC RECEPTORS ON INTACT S49 LYMPHOMA CELLS. E. E. Reynolds*, D. R. Hoyer*, and P. B. Molinoff. Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104.

The binding of (^{125}I)-iodopindolol (IPIN) to β-adrenergic receptors was characterized in intact S49 lymphoma cells maintained in suspension culture. Binding was stereospecific, saturable, reversible, and stable for up to 90 minutes at 37° C. The binding of IPIN reached equilibrium rapidly, allowing quantitation of receptor properties after short periods of incubation. Experiments were carried out in which cells were incubated with IPIN and various concentrations of competing drugs. The affinity of the receptor for isoproterenol changed from a K_D of less than 1 μM after a 1 minute incubation to a K_D of 20-50 μM measured at equilibrium after a 30 minute incubation. This time-dependent shift in affinity was agonist-specific in that similar effects were not observed in studies of the interactions of antagonists with receptors on intact S49 cells. In kinetic experiments in which inhibition of the binding of IPIN was studied, 5 μM isoproterenol inhibited almost all of the binding of IPIN at early time points (1-2 min.), but had very little effect at equilibrium (30 min.). In addition, IPIN binding reached equilibrium more rapidly in the presence of isoproterenol, suggesting that an agonist-induced increase in the affinity of the receptors for antagonists occurs. Studies of agonist inhibition curves of IPIN binding, as carried out in intact cells, were analyzed by non-linear regression analysis. A highly significant improvement of the fit was observed when the data was modeled for two affinity states of agonist-receptor complex rather than one state. The results are consistent with the hypothesis that the properties of some but not all of the receptors were affected by the presence of isoproterenol. These observations with regard to the properties of β-adrenergic receptors on intact S49 cells are generally similar to those previously observed in studies of adrenergic receptors on intact L6 muscle cells (J. Cyc. Nuc. Res., 6: 421, 1980). However, time-dependent shifts in agonist affinity were only seen when L6 cells were attached to the surface of the incubation dish and not after they had been detached from the dish. In contrast, S49 cells exhibit this effect both in suspension and when attached to poly-L-lysine coated dishes. Furthermore, agonists cause all of the receptors on L6 cells to shift to a form with a low affinity for agonists. In studies with S49 cells two forms of the receptor were seen when assays were carried out in the presence of an agonist. Studies of the properties of β-adrenergic receptors on intact cells suggest that there are important regulatory mechanisms that are seen only in intact cells and are lost upon homogenization of the cells. IPIN appears to be a useful ligand to study these phenomena because it allows precise quantitation of changes in receptor properties at short incubation times. S49 cells provide a potentially useful system with which to study these effects because it is possible to perform similar studies in S49 variants which either lack the guanine nucleotide binding regulatory protein (G/F) or in which the receptor and G/F are uncoupled. (Supported by the USPHS NS 18479).

187.2 ALPHA$_2$ ADRENERGIC ACTIVITY IN THE INTACT HUMAN PLATELET: RELATIONSHIP BETWEEN RECEPTOR BINDING AND INHIBITION OF ADENYLATE CYCLASE. R.H. Lenox, J. Ellis*, D. VanRiper*, Y.H. Ehrlich. Dept. Psychiatry Univ. Vermont College of Medicine, Burlington, VT 05405.

The alpha (α) adrenergic receptor of the human platelet possesses pharmacological characteristics consistent with an α_2 subtype, mediating inhibition of prostaglandin-stimulated adenylate cyclase (AC). Our laboratory has established assay conditions for the characterization of concentration dependent norepinephrine (NE) inhibition of prostacyclin (PGI_2) stimulated AC in intact human platelets prelabeled with ^{3}H adenine (Lenox et. al Neurosci. Abst. 6: 252, 1980). We have shown that NE acts as a full agonist inhibiting 85% of the PGI_2-stimulated AC activity at saturating concentrations, whereas clonidine (CL) acts as a partial agonist, maximally inhibiting only 35% of the same activity. Yohimbine (YOH) will completely reverse both NE and CL inhibition of AC activity, while CL only partially reverses NE inhibition (back to the 35% level).

We have investigated the relationship between binding characteristics of the α_2 radioligands and the inhibition of AC activity produced by these ligands in the intact cell. Since the properties of agonist binding have been shown to be extremely sensitive to ions and nucleotides, and characteristics of receptor binding in membranes vs intact cells can differ considerably, our binding assays have been conducted under the same conditions employed in the cyclase assays. Saturation of ^{3}H YOH (80-90 Ci/mmol) binding in intact platelets under these conditions followed single site kinetics (K_D 4.24 ± .25nM and Bmax 134 ± 15 sites/platelet). The occupancy of NE, E and CL was inferred from the concentration dependent inhibition of ^{3}H YOH binding carried out in parallel with concentration dependent inhibition of PGI_2 stimulated AC.

	Binding K_I (μM) ± SEM	Cyclase EC_{50} (μM) ± SEM	Ratio K_I/EC_{50}
NE	19.0 ± 5.3	0.65 ± .05	29.2
E	5.2 ± 1.0	0.24 ± .08	21.7
CL	0.24 ± .04	0.20 ± .04	1.2

The comparable affinities of CL in both assay systems stands in contrast to the relative affinities for NE and E, and is consistent with its pharmacological role as a partial agonist. We have initiated a series of investigations to address the relatively lower affinity observed for NE and E in the receptor binding assays. Preliminary experiments have indicated that NE inhibition of AC activity can remain effectively intact following a preincubation with the irreversible antagonist phenoxybenzamine which significantly reduces the specific binding of ^{3}H YOH. These data will be discussed in light of current concepts related to proposed multiple affinity states of the α_2 receptor and the possibility of spare receptors in the intact human platelet. Supported in part by PHS 5429-18-3 and Upjohn Company.

187.3 EFFECTS OF COMBINED ADMINISTRATION OF IMIPRAMINE AND CHLORPROMAZINE ON BETA-ADRENORECEPTORS IN RAT CEREBRAL CORTEX. M. Mikuni and H.Y. Meltzer. Dept. of Psychiat., Univ. of Chicago Pritzker School of Medicine, Chicago, IL. 60637

It has been well established that a variety of antidepressant drugs cause down regulation of β-adrenergic receptors in the rat brain, leading to the hypothesis that the therapeutic effects of these drugs may be related, in part, to their ability to decrease central β-adrenergic receptor sensitivity. There is also extensive clinical evidence that combined administration of antidepressant and neuroleptic drugs is necessary and sufficient pharmacotherapy for patients with delusional depression who fail to respond to either drug alone. In this study, we investigated the effects of combined administration of imipramine and chlorpromazine on β-adrenergic receptors in rat cerebral cortex.

Adult male Sprague-Dawley rats (200 g) were treated with either imipramine (Imip) (10 mg/kg, ip) or chlorpromazine (CPZ) (5 mg/kg, ip) or both, twice daily for 14 days. Control animals received the same volume of saline. All animals were sacrificed by decapitation 22 hrs after the last injection. The brains were immediately removed, dissected and frozen. Membranes of cerebral cortex were prepared as described previously in 50 mM Tris buffer (Bylund and Snyder, 1976). Incubation of membrane preparation (5 mg original wet weight) with ^{3}H-DHA (0.1-4.0 nM) was carried out in a final volume of 1.0 ml for 30 min at 25°C; specific binding was defined by 100 μM (-)-isoproterenol. Imip alone and Imip plus CPZ reduced the number of β-adrenergic receptor binding sites in the cerebral cortex (35%) as compared to saline controls, but there was no significant change in Bmax or K_D after treatment with CPZ alone.

These results indicate that there is no interaction between CPZ and Imip with regard to down regulation of β-adrenergic receptors. It is possible that the greater efficacy of the continuation of these two agents clinically represents the combination of their independent effects upon beta-adrenergic and dopamine receptors. Investigation of the effect of the combined treatment on α_2 and 5-HT_2 receptors are in progress since imipramine alone has been reported to decrease the number of these receptors in brain.

187.4 ^{3}H-RAUWOLSCINE AND ^{3}H-PARA-AMINOCLONIDINE: AUTORADIOGRAPHIC ANALYSIS OF THE DISTRIBUTION OF ALPHA-2 BINDING SITES LABELED WITH ANTAGONISTS OR AGONISTS. J.R. Unnerstall, T.A. Kopajtic* and M.J. Kuhar. Dept. Neurosci., Johns Hopkins Univ. Balto., MD 21205

^{3}H-Clonidine and ^{3}H-para-aminoclonidine (^{3}H-PAC) have been used as agonist ligands to study alpha-2 adrenergic receptors. ^{3}H-Rauwolscine (^{3}H-RW) is presently the most specific alpha-2 antagonist ligand available, and its binding in brain homogenates has recently been characterized (Perry and U'Prichard, Eur. J. Pharmacol. 76:461, 1981). We have characterized the binding of ^{3}H-RW in intact sections of rat brain processed for light microscopic autoradiography (LMA) and have compared the distribution of binding sites labeled with ^{3}H-RW or ^{3}H-PAC.

In 50 mM K^+-PO_4 buffer containing 50 mM NaCl (pH.7.4) at room temperature, the binding of ^{3}H-RW was comparable to that described by Perry and U'Prichard (see above). However, dissociation at 4°C was biphasic and Scatchard analysis of saturation experiments revealed curvilinear plots with approximate K_D's of 1 and 6 nM for the high and low affinity components respectively. Scatchard plots of ^{3}H-PAC binding in Tris-HCl buffer (pH7.6) with the addition of 10 mM $MgCl_2$ at room temperature were also biphasic with K_D's of 0.25 and 2.5 nM. For LMA, serial coronal sections were incubated in the presence of either 3 nM ^{3}H-RW or 1 nM ^{3}H-PAC. Nonspecific binding was defined in the presence of 10 uM phentolamine. Under these conditions, 70-80% of the high-affinity sites and 25-35% of the low affinity sites will be occupied for each ligand.

In general, autoradiograms generated by these two ligands revealed similar binding site distributions in the rat brain (see Young and Kuhar, PNAS 77:1696, 1980). However, in some regions, such as the superficial lamina of CA1 of the hippocampus, the molecular and polymorphic layers of the dentate gyrus, the dorsal parabrachial nucleus and the ventro-lateral reticular formation, ^{3}H-RW binding appeared higher than that of ^{3}H-PAC. Especially striking were the high levels of ^{3}H-RW binding seen in the striatum and the substantia nigra pars compacta and lateralis which are not seen with ^{3}H-PAC.

Since saturation experiments reveal two binding sites for both ^{3}H-RW and ^{3}H-PAC and since ligand concentrations were chosen to produce comparable receptor occupancies for the multiple ^{3}H-RW and ^{3}H-PAC sites, the differences seen by LMA in the distribution of ^{3}H-RW and ^{3}H-PAC binding sites may reflect a differential selectivity of these two ligands for the two states of the alpha-2 receptor. However, these data cannot rule out the possibility that ^{3}H-RW also labels a receptor other than the alpha-2 receptor at the ligand concentrations used in these experiments. Additional work is in progress to explore these questions.

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187.5 THE BINDING OF ^{3}H-WB4101 AND ^{3}H-PRAZOSIN DIFFERS IN THE HIPPOCAMPUS OF THE RAT. J.J. Valdes, J.R. Unnerstall and M.J. Kuhar. Tox. Branch-Chem. Systems Lab, Aberdeen Proving Grounds, MD and Dept. of Neurosci., Johns Hopkins Sch. Med., Balto., MD 21205

^{3}H-WB4101 (^{3}H-WB) and ^{3}H-prazosin (^{3}H-PRZ) have been used to label alpha-1 binding sites. However, several published studies have shown that PRZ is much more selective than WB as an alpha-1 ligand and antagonist. We have seen differences in the distribution of binding sites labeled with either ^{3}H-WB or ^{3}H-PRZ in the rat brain using light microscopic autoradiography (LMA). For example, ^{3}H-WB binding is high and uniform in the hippocampus and dentate gyrus. ^{3}H-PRZ binding is low in these regions. In order to study this difference, we have compared the characteristics of ^{3}H-WB and ^{3}H-PRZ binding in rat hippocampal membranes.

In preliminary experiments in rat neocortical membranes, we discovered that in 50 mM Na^+-K^+-PO_4 buffer (pH7.4) the K_D's for both ^{3}H-WB and ^{3}H-PRZ were 2-3 times lower than those seen when binding was done in 50 mM Tris-HCl buffer (pH7.4). This effect was not due to the presence of Na^+ ions, and no change in the B_{max}'s was observed. In subsequent experiments, binding was done in the PO_4 buffer at 25°C for 60 min. Tissue concentration was 1 mg wet wt per ml incubation (final volume 5 ml). Nonspecific binding was defined in the presence of 10 uM phentolamine.

In the hippocampus, ^{3}H-PRZ binding was biphasic with K_D's of 0.07 and 1.6 nM and a total B_{max} of 15 fmole/mg tis. Binding to the high-affinity site represented 30% of the total binding. ^{3}H-WB also labeled two sites: a high-affinity site with a K_D of 0.2 nM and a B_{max} of 13 fmole/mg tis. and a second site of much lower affinity and high capacity. PRZ displacement of 0.2 nM ^{3}H-WB was biphasic with a broad plateua between 1 and 100 nM PRZ. Approximately 50% of the ^{3}H-WB binding was competitively displaced by 100 nM PRZ. The binding to the low-affinity site was not affected by this concentration of PRZ.

To summarize, ^{3}H-WB appears to label two populations of binding sites in the hippocampus. The high affinity ^{3}H-WB site is displaced by PRZ with high affinity and is probably the same site labeled with high-affinity by ^{3}H-PRZ. The ^{3}H-WB site of much lower affinity is displaced by PRZ only at uM concentrations. This latter site, although pharmacologically uncharacterized, is concentrated in the hippocampus and has characteristics similar to the ^{3}H-WB site seen in the periphery. These observations can account for the difference in the density of ^{3}H-WB and ^{3}H-PRZ binding seen by LMA in this region. Further, these results support previous studies which showed that ^{3}H-WB was not a selective alpha-1 ligand and indicated that ^{3}H-WB may not be the ligand of choice in studying the alpha-1 even in the central nervous system.

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187.6 IMPAIRED RECOVERY OF BRAIN β-ADRENERGIC RECEPTORS IN AGED RATS FOLLOWING DESMETHYLIMIPRAMINE-INDUCED SUBSENSITIVITY, L. H. Greenberg, D. Brunswick and B. Weiss, Dept. of Pharmacology, Med. Coll. of Pa., Phila. 19129 & Neuropsychopharmacology Unit, Vet. Admin. Hosp., Phila., PA 19104

Repeated but not acute administration of the tricyclic antidepressant desmethylimipramine (DMI) produced subsensitivity of β-adrenergic receptors in the cerebral cortices and pineal glands from young (3-mo) and aged (24-mo) rats. However, brain tissues from aged rats showed an impaired ability to increase β-receptor density in response to reduced noradrenergic input (Greenberg & Weiss, J. Pharmacol. Exp. Ther. 211:301, 1979). In the present study we compared the ability of young and old rats to restore their density of β-receptors following DMI-induced receptor subsensitivity. DMI was administered twice daily for seven days to 2 groups of young rats (2-3mo) at a dose of either 5 or 10mg/kg, i.p., and to 1 group of aged rats (20-26mo) at a dose of 5mg/kg. Control rats of each age received saline for the same time period. Rats from each group were decapitated 4 hr and 1,2,4 and 8 days after the last DMI or saline injection. [^{3}H]dihydroalprenolol (DHA) binding was determined in homogenates of pineal gland and cerebral cortex, and DMI concentrations in serum and cortex were determined by radioimmunoassay. DMI produced a significant decrease in DHA binding in both brain areas of young & aged rats 4 hr after the last dose of DMI. In young rats, DHA binding in the pineals and cortices returned to control levels by 2 days after the last dose of DMI. In contrast, in aged rats it took 4 days for DHA binding to return to control values in the cortex and more than 8 days to return to normal in the pineals. The serum concentration and half-life ($t_{\frac{1}{2}}$) of DMI in the aged rats was significantly greater than that in the young rats at both drug doses (e.g., $t_{\frac{1}{2}}$ at the 5mg/kg dose was approximately 0.5 days in the young and 1.7 days in the aged rats). Similar differences between the groups were found for the $t_{\frac{1}{2}}$'s of DMI in cortex. Although the pharmacokinetic properties of DMI were altered in the aged rats, these differences were not sufficient to account for the delayed recovery of β-receptors in aged rats. These results suggest that one must beware of the age-related alterations in the pharamcokinetic properties of CNS-acting drugs when conducting studies of these agents in the aged. The results also suggest that brain tissue from aged rats cannot recover from β-receptor subsensitivity as readily as that from young rats. If this recovery process requires the synthesis of new receptors, then this synthetic mechanism may be impaired with age. Supported by Grants NS12642 and MH30096 and funds from the Veterans Administration.

187.7 ROLE OF AMINERGIC NEURONAL INPUT IN THE DOWN-REGULATION BY DESIPRAMINE (DMI) OF THE NOREPINEPHRINE (NE) RECEPTOR COUPLED ADENYLATE CYCLASE SYSTEM IN RAT CORTEX. F. Okada*, D.H. Manier*, A.J. Janowsky*, L.R. Steranka and F. Sulser. Vanderbilt University School of Medicine, Nashville, TN 37232 and Indiana University, Gary, Indiana 46408.

Previous studies have shown that the ability of antidepressants to elicit subsensitivity of the NE sensitive adenylate cyclase system linked to a reduction in the density of β-adrenoceptors depends on an intact noradrenergic neuronal input. In the present investigations, we studied the consequences of selective lesioning of serotonergic neurons with 5,7-dihydroxytryptamine (5,7-DHT) on the down-regulation by DMI of the NE receptor coupled adenylate cyclase system in cortex from normal animals and from animals with an increased density of β-adrenoceptors (d,l-propranolol, 10 mg/kg b.i.d. for 7 to 14 days). In agreement with results obtained by Brunello et al. (In: New Vistas of Depression, S.Z. Langer, B. Briley, eds., Pergamon Press, New York, in press, 1982), DMI failed to decrease the density of β-adrenoceptors (^{3}H-dihydroalprenolol binding) in the absence of serotonergic input (B_{max} 131 ± 4 and 133 ± 25 fmol/mg protein ± SEM in control and DMI treated animals respectively). However, DMI reduced the response of the cyclic AMP generating system to the β-agonist isoproterenol (10 μM) in lesioned and control animals to the same degree (from 67 ± 11 to 39 ± 6 and from 63 ± 9 to 30 ± 5 pmol cyclic AMP/mg protein ± SEM in control and 5,7-DHT lesioned animals respectively). In animals whose β-adrenoceptor population was up-regulated by propranolol, DMI failed to reduce both the sensitivity of the system to agonists and the B_{max} value of β-adrenoceptors. In fact, both the cyclic AMP responses and the B_{max} value of ^{3}H-DHA binding were further increased by DMI. Selective lesioning with 5,7-DHT had no influence on the up-regulation of the receptor system by propranolol. Moreover, the differentiation in the effects of DMI on recognition and action function of the receptor system did not occur in lesioned animals treated with propranolol. The results provide direct evidence for a complex functional linkage between serotonergic and noradrenergic neuronal systems at the molecular level indicating that serotonergic neuronal input is co-required for the regulation of the β-adrenoceptor moiety of the receptor coupled adenylate cyclase system but not for the induction by DMI of subsensitivity to isoproterenol. Studies are now in progress to elucidate the differences in the mechanism of the regulation by DMI of β-adrenoceptors and their coupling to adenylate cyclase following lesions with 5,7-DHT in normal and propranolol treated animals. (Supported by USPHS Grant MH-29228, the Tennessee Dept. of Mental Health and Mental Retardation and the Lake County Medical Center Developmental Agency, Lake County, Indiana).

187.8 MODULATION BY SODIUM, LITHIUM AND CALCIUM OF SPECIFIC 3H-PRAZOSIN BINDING IN NEOCORTEX. T.A. Reader and R. Brière*. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3T8.

The use of binding assay techniques for the study of neurotransmitter-receptor interactions has now been well established and the recent introduction of ^{3}H-labelled Prazosin (PRZ; an α_1-antagonist) has enabled the determination of some of the properties of cortical postsynaptic receptors (1-3). In the majority of these in vitro studies the buffers used were made of Tris-HCl and in some cases Mg^{++} was added (4). This saline composition is quite unlike the normal ionic environment found in the CNS. As a first approach to obtain more physiological conditions for binding experiments, we investigated the effects of Na^+ (5), Li^+ and Ca^{++}, and established the best incubation conditions for ^{3}H-PRZ binding in ionic media which reflect more accurately the brain's ionic environment. Specific ^{3}H-PRZ (0.5 nM) binding to neocortical membrane preparations increased linearly (r= 0.990) with increasing protein concentration (from 142 to 1.721 μg/ml) (3,4). This binding reached equilibrium (25°C) at 25-30 min, and was maintained up to 60 min. Half saturation (0.5 nM) was obtained in 7.5 min. After equilibrium was reached, specific binding could be displaced by PRZ or phentolamine. Saturation experiments (0.1 to 5 nM) revealed one high affinity site and from the Scatchard-Rosenthal analysis a Bmax of 120 fmol/mg protein with a KD 25°C of 0.686 nM were derived (Hill number of 0.88). Increasing the concentration of Na^+ (0-200 mM) specific binding increased (but only in the presence of 10 mM $MgCl_2$). The optimal Na^+ concentration was established at 150 mM. Similarly, the effect of Ca^{++} (in the absence of Na^+) was to increase specific binding, but only if Mg^{++} was present. The optimal concentration for the Ca^{++} effect was determined at 1.4 mM. When both Na^+ (150 mM) and Ca^{++} (1.4 mM) were added to the incubation buffer, the affinity increased (KD 25°C of 0.123 nM) without any change in Bmax (Hill N= 0.997). In the presence of Na^+ alone the KD 25°C was of 0.414 nM (Hill N= 0.920). When Na^+ was replaced by Li^+ (in Ca^{++} 0 mM) there was a ten-fold decrease in the affinity (KD 25°C= 1.1nM) without changes in Bmax. These preliminary experiments show that ^{3}H-PRZ binding assays can be performed in buffers that approach the physiological salt-composition of brain tissue and indicate the participation of ions in the complex interactions between ligands and receptor sites. References: 1) Miach, P.J. et al, Nature 274 (1978) 492; 2) Greengrass, P. and Bremner, R., Europ. J. Pharmacol. 55 (1979) 323; 3) Hornung, R. et al., Naunyn-Schmiedeberg's Arch. Pharmacol. 308 (1979) 223; 4) Miach, P.J. et al., Naunyn-Schmiedeberg's Arch. Pharmacol. 312 (1980) 23; 5) Pert, C.B. and Snyder, S.H., Molec. Pharmacol. 10 (1974) 868. [Supported by the MRC (Canada) and the Canad. Heart Found.].

187.9 CIRCADIAN RHYTHMS IN ALPHA- AND BETA-ADRENERGIC RECEPTOR NUMBER AND CYCLIC AMP PRODUCTION IN RAT CEREBRAL CORTICAL SLICES. M.A. Benedito* and M.S. Kafka. Neuroscience Branch, NIMH, Bethesda, MD 20205.

Circadian rhythms in neurotransmitter receptors (α- and β-adrenergic, cholinergic, dopamine, opiate, and benzodiazepine) have been demonstrated in rat brain. Noradrenergic agonist binding to α- and β-adrenergic receptors in the brain stimulates the activity of an adenylate cyclase (Daly et al., J. Neurosci., 1:49-59, 1981). The stimulated synthesis of cyclic AMP (cAMP) then, can be used as a physiological measure of receptors in the membrane. Male rats were entrained for 3 wk to a controlled light-dark cycle (L:D, 12:12) and enucleated 56 hr before sacrifice to eliminate time cues from the light:dark cycle. Groups of 18 rats were sacrificed every 4 hr over a 24-hr period, brains removed, cortices dissected and sliced. Cyclic AMP was measured by the conversion of ^{3}H-ATP to ^{3}H-cAMP. Alpha- and β-receptors were measured in membranes from frozen slices by the specific binding of ^{3}H-WB4101 with and without phentolamine, and ^{3}H-dihydroalprenolol (^{3}H-DHA) with and without dl-propranolol, respectively. Alpha-adrenergic receptor binding varied during the course of the day, increasing from a trough at 6H to peak at 14H. Alpha-receptor stimulated ^{3}H-cAMP accumulation, i.e., l-norepinephrine (NE)-stimulated ^{3}H-cAMP accumulation in ^{3}H-adenine-labelled cortical slices in the presence of sufficient dl-propranolol to block the β-receptors, increased from a trough at 6H to a peak at 10H. Beta-adrenergic receptor binding peaked at 14H and 2H with a trough at 18H. Beta-receptor-stimulated ^{3}H-cAMP accumulation, i.e., NE-stimulated ^{3}H-cAMP in presence of sufficient phentolamine to block the α-receptors, peaked at 14H with a trough at 18H. Combined α- and β-receptor-stimulated cAMP accumulation, i.e., ^{3}H-cAMP accumulation induced by NE measured in the absence of antagonists, peaked at 14H and 2H with a trough at 22H. Scatchard analysis of α- and β-adrenergic receptor saturation isotherms indicated no changes in the affinity of either receptor at times of maximal and minimal binding in slices. A change in specific binding is then, a change in the number of receptors. In slices from rat cerebral cortex α- and β-adrenergic receptor number and ^{3}H-cAMP formation due to the activation of α- and β-adrenergic receptors have similar circadian rhythms. The data suggest that the circadian changes in cAMP production are a cellular response to the circadian changes in α- and β-receptor number. The circadian rhythms in α- and β-receptor number could alter neuronal activity in the brain over the course of the day.

187.10 BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF ADRENERGIC RECEPTORS ON EMBRYONIC SENSORY NEURONS. K. Dunlap and D.R. Canfield*. Dept. of Physiology, Tufts Univ. Sch. of Med., Boston, MA 02111.

Nor-epinephrine (NE) decreases the voltage-dependent Ca current in the soma membrane of embryonic chick sensory neurons (Dunlap and Fischbach, 1981). This effect is mediated by an α-adrenergic receptor: phentolamine, but not propranolol, blocks the response to NE. The NE-induced decrease in Ca current is also blocked by yohimbine (ED_{50} = 3.2 nm) suggesting that the receptor may be classified as α_2. To begin to characterize this receptor biochemically standard filtration binding techniques were employed using ^{3}H-yohimbine (New England Nuclear). Crude membranes were prepared from embryonic chick sensory neurons grown in the absence of other cell types in dissociated cell culture. Cells were homogenized in 50 mM Tris-10 mM $MgCl_2$ buffer, centrifuged 10 min at 15,000 x g, washed once in the same buffer, repelleted and suspended in buffer at a concentration of 1-2 mg protein/ml. Membranes were incubated 10 min at 37°C with the radioactive ligand ± excess cold yohimbine and then washed in a filtering manifold with 40 ml buffer. Specific binding (that displaceable by cold yohimbine) saturated at ca. 10 nM with an apparent K_D of 2.4 nM. At the K_D specific binding was approximately 50% of total binding. Maximum specific binding ranged between 40 and 80 fmole/mg protein in membranes prepared from different platings. ^{3}H-yohimbine binding was inhibited by NE with an ID_{50} ranging between 0.5 and 1.6 μM which agrees well with the ED_{50} for the NE-induced decrease in Ca current (0.6 μM). Attempts are now being made to observe ^{3}H-yohimbine binding in whole cells to allow the use of autoradiographic techniques to determine the distribution as well as density of these α-adrenergic receptors on the cell surface.

Preliminary evidence suggests that the NE-induced decrease in Ca current may involve a second messenger. When bath applied at 1 mM, monobutyryl cAMP mimicked the effect of NE in decreasing the sensory neuron action potential duration. In addition, this compound also potentiated the magnitude of the NE response when the two were applied together. IBMX (at 1 mM) alone decreased action potential duration when bath applied. Dibutyryl cAMP, dibutyryl cGMP and monobutyryl cGMP all were ineffective (at 1 mM or 10 mM).

Dunlap, K. and G.D. Fischbach, (1981) J. Physiol. 317: 519-535.
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187.11 CATECHOLAMINE RECEPTOR AGONIST EFFECTS ON CYCLIC AMP PRODUCTION IN STRIATAL SLICES. G.G. Leblanc*, R.E. Boehme* and R.D. Ciaranello. Dept. of Psychiatry, Stanford Univ. Sch. of Med., Stanford, CA. 94305.

Cyclic AMP is thought to act as a second messanger for catecholamines in the striatum. Dopamine (DA) and norepinephrine (NE) have previously been found to stimulate cAMP production in homogenates and intact slices of rat striatum. The identity of the receptor types which mediate the stimulation remains uncertain, and is to some extent dependent on the tissue preparation used. The present studies attempt to clarify the pharmacology of catecholamine regulation of cAMP levels in striatal slices, and to examine potential interactions between different receptor types.

Male rats were sacrificed, and the striata were dissected on ice and cut into 450μ slices on a McIlwain tissue chopper. Slices were incubated in Hepes-buffered Krebs salts, pH 7.35-7.4, under 95% O_2-5% CO_2 in an agitating water bath at 35°C. After one hour of preincubation, drugs were added and the slices were incubated for an additional 10 minutes. Following extraction in boiling .01N HCl, the cAMP content of the slices were assayed by a modification of the Gilman protein binding assay.

In striatal slices, maximally effective concentrations of isoproterenol (ISO) (10 μM) and NE (300 μM) stimulated cAMP to 300 and 1200% of control levels, respectively. The response to 300 μM NE was inhibited 90% by propranolol (10μM), while phentolamine (10 μM) and cis-flupenthixol (10 μM) each inhibited the response by 50%. DA and the alpha agonist 6-fluoronorepinephrine (6FNE) had no effect on cAMP levels at concentrations up to 300 μM. However, 300 μM DA or 50 μM 6FNE potentiated the ISO response approximately two-fold. These results contrast with those obtained in comparative studies of adenylate cyclase in striatal homogenates. Both DA (30 μM) and 6FNE (100 μM) stimulated adenylate cyclase activity by 100%, while isoproterenol (30 μM) was virtually inactive.

It is difficult to determine pharmacologically whether the actions of DA and 6FNE are mediated by an alpha-adrenergic receptor, a D-1 type dopamine receptor, or both. DA and 6FNE are alike in that both agonists appear to stimulate adenylate cyclase directly in striatal homogenates. Futhermore, our results suggest that stimulation of cAMP levels in striatal slices by both DA and 6FNE require concomitant beta-receptor stimulation. Hence, alpha- and DA-receptor agonists may differ from beta agonists in the mechanism by which they increase cAMP levels in striatal slices.

187.12 MOUSE STRAIN DIFFERENCES IN CEREBRAL CORTICAL BETA-ADRENERGIC RECEPTOR SUPERSENSITIVITY. J.A. Severson, C.E. Finch, R.N. Pittman and P.B. Molinoff. Univ. So. Calif., Sch. of Med., Depts. Psych. and Physiol., Los Angeles, CA 90033, Univ. Penn., Sch. of Med., Dept. Pharmacol., Philadelphia, PA 19174.

CBA/J mice, compared to BALB/cJ mice demonstrate many differences in the nigro-striatal pathway, such as fewer nigral and striatal neurons, lower nigral and striatal tyrosine hydroxylase activity and fewer striatal dopamine (DA) receptors. Additionally, CBA/J mice do not develop striatal DA receptor supersensitivity to chronic haloperidol treatment. This study was conducted to determine if the absence of receptor supersensitivity in CBA/J mice is limited to the striatal DA receptor or if the impaired response is generalized to monoamine receptors in other brain regions.

Male CBA/J, C57BL/6J and BALB/cJ mice received systemic infusions of 10 ug propranolol/hr for 1 wk via Alzet minipumps. Mice were sacrificed the day following removal of the pump and the cerebral cortex dissected for the determination of beta-adrenergic receptor subtypes by ^{125}I-HYP binding and competition with zinterol.

Cerebral cortical ^{125}I-HYP Bmax in BALB/cJ mice was 25-30% of that in CBA/J and C57BL/6J. BALB/cJ also had the lowest proportion of the beta-1 subtype (86%) vs 90% for C57BL/6J and 94% for CBA/J. Beta-2 receptors were similar in all strains.

Propranolol increased Bmax in all strains, but CBA/J's had the smallest increase (24%). BALB/cJ mice showed a marked increase in the number of beta receptors (192%) and C57BL/6J were intermediate (42%). In all strains the increase in beta receptors was limited to the beta-1 subtype.

These data indicate that the impaired supersensitivity response in CBA/J mice is not restricted to the striatal DA receptor and suggests a genetic influence on supersensitization that may be extended to other monoamine receptors. The enhanced supersensitivity of BALB/cJ mice to propranolol suggests that the extent of responsiveness to beta blockers is under genetic influence.

187.13 AGE RELATED DIFFERENTIAL REGULATION OF RAT CORTEX ADRENERGIC RECEPTORS DUE TO INNERVATION STATUS. C. H. Wang and D. C. U'Prichard. Neuroscience Graduate Program and Dept. Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Rat fetus exposed to a potent antimitotic agent methylazoxymethanol acetate (MAM) at gestation day 15 results in frontal microencephaly and adrenergic hyperinnervation (Johnston and Coyle 1979). We examed the effects of this congenital hyperinnervation on cerebral cortical β-, α_1-, and α_2-adrenergic receptors labeled with ^{3}H-dihydroalprenolol (DHA, β), ^{3}H-prazosin (PRAZ, α_1), ^{3}H-P-aminoclonidine (PAC, α_2H), ^{3}H-yohimbine (YOH, α_2L), and ^{3}H-rauwolscine (RAUW, α_2L). Previously, we reported that decrease in Bmax of all adrenergic receptor subtypes were observed at 10-16 weeks of age compared with the age-matched normal controls. In the present study, we observed differential alterations of these receptor subtypes at 20 weeks of age. β- and α_1-R Bmax values remained decreased (-26% for DHA and -10% for PRAZ) but α_2-R showed significant rebound increase compared with age-matched controls (+30% for PAC, +52% for YOH and +23% for RAUW). This increase in α_2-R were also observed at 30 weeks of age with similar magnitudes of Bmax alteration for the three α_2-ligands. All receptor assays revealed no significant change in Kd values. Further characterization of the altered receptors using competition of ^{3}H-antagonists by unlabeled agonists showed no difference between the MAM and control groups measured at 10 or 20 weeks of age indicating no selective change in affinity of certain receptor populations.

Intraventricular 6-hydroxydopamine (6-OHDA) administration to 30 week old MAM-treated and normal control rats resulted in early decrease (-20% to -30% observed 1 week after lesion) and subsequent increase (+20% to +40% observed 4 weeks after lesion) in ^{3}H-PAC and ^{3}H-YOH Bmax values in both 6-OHDA groups. The MAM-treated cortex has apparently different α_2-receptor plasticity compared to normal cortex in that it exhibits a more modest and retarded increase in responding to the presynaptic 6-OHDA lesion. These results suggest that rat cortex adrenergic receptors undergo chronic regulation by the presynaptic NE input and may vary in their homeostatic capacity in the congenital hyperinnervated state through different ages. The decrease in α_2-R observed soon after the 6-OHDA lesion may indicate labeling of presynaptic α_2-R in rat cortex. (Supported by USPHS grant NS 15595).

187.14 ION AND NUCLEOTIDE REGULATION OF α_1 RECEPTOR BINDING IN RAT KIDNEY P. Ernsberger and D.C. U'Prichard. Neuroscience Program and Dept. of Pharmacology, Northwestern University, Chicago, IL 60610

Previous studies have shown that monovalent cations and guanine nucleotides decrease and divalent cations increase the affinity of agonists in a number of receptor systems linked to adenylate cyclase. The α_1 receptor, selectively labeled with ^{3}H-prazosin (PRAZ), is generally assumed not to be coupled to adenylate cyclase in most tissues, and thus to be insensitive to mono- and divalent cations and guanine nucleotides. In the present study, PRAZ binding was examined in the rat kidney. Renal cortex or medulla was homogenized in 50mM Tris-HCl buffer, pH 7.7, containing 5mM EDTA. The supernatent from a 300g centrifugation was pelleted twice at 50,000g in Tris-EDTA, and then washed with Tris without EDTA. The final suspension in Tris was added to tubes containing 300pM PRAZ, with and without 10μM phentolamine (phent) as a blank, or varying concentrations of competing drugs, and incubated for 30 min. at 25C° prior to rapid filtration. Results for cortex and medulla were generally similar. Norepinephrine (NE) displaced PRAZ with a K_i of 410nM and a Hill slope (n_H) of 0.55, indicating a heterogeneous interaction. 100mM NaCl increased the K_i of NE about 2-fold (226±27% of control), as well as the n_H of NE (0.83 vs. 0.55). Na also decreased the K_D of PRAZ (186 vs. 261pM), while the B_{max} was unchanged (126 vs. 136 fmol/mg protein). Li had similar effects on PRAZ binding and NE competition, while K and Cs were inactive. Na caused no effect or a slight decrease in the K_i for the antagonists phent and WB4101. The effect of Na on NE competition reaches a maximum around 10mM NaCl, while the enhancement of PRAZ binding continues to rise up to 100mM. 5mM $MgCl_2$ decreased the K_i of NE (72±13% of control), without affecting n_H. Mg also increased the K_D of PRAZ (370 vs. 261pM), with B_{max} unchanged. Na reversed the Mg effect on both PRAZ binding itself and NE competition for PRAZ sites. 100μM GTP or Gpp(NH)p, but not ATP or GMP, increased the K_i of NE (200-400% of control). However, 100μM GTP had no effect on PRAZ saturation (K_D: 237 vs. 261pM). GTP had no effect on the K_i of phent and WB4101. GTP and Na effects were not additive. In summary, mono- and divalent cations and guanine nucleotides had effects on agonist competition at rat kidney α_1 sites labeled with PRAZ similar to those previously observed at cyclase-coupled α_2 sites. Ion and nucleotide regulation of α-adrenergic receptors will be examined in the kidneys of Dahl salt-sensitive rats, which display abnormalities in receptor number as well as the response to ions in vivo. (Supported by an American Heart Assoc. grant-in-aid, and an NSF Predoctoral Fellowship to P.E.)

188.1 IMMUNOCYTOCHEMICAL LOCALIZATION OF CHOLINERGIC NEURONS WITH A MONOCLONAL ANTIBODY TO CHOLINE ACETYLTRANSFERASE. C.R. Houser, G. Crawford*, L. Anderson*, R. Barber*, P.M. Salvaterra and J.E. Vaughn. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Choline acetyltransferase (ChAT), a specific marker for cholinergic neurons, has been localized immunocytochemically with a monoclonal antibody in light and electron microscopic preparations of rat CNS. The antibody is an IgG_1 subclass immunoglobulin that selectively removes ChAT activity from solution (Crawford, G. and Salvaterra, P.M., Trans. Am. Soc. Neurochem., 13:104, 1982).

Putative cholinergic systems were studied to test the specificity of the antibody and the method. In spinal cord and brain stem motor nuclei, the somata, dendrites and axons of motoneurons were stained for ChAT. ChAT-positive puncta also were present, and EM studies indicated that some of these structures were synaptic terminals. In the septo-hippocampal system, many stained somata were observed in nuclei of the medial septum and diagonal band. ChAT-positive fibers were present in the fimbria, and presumptive axon terminals were stained throughout the hippocampal formation. They were most numerous immediately superficial and deep to strata granulosum and pyramidale. In the habenulo-interpeduncular system, ChAT-positive somata were present in the medial habenular nucleus, and numerous terminal-like structures were stained in the interpeduncular nucleus. In all of these regions, ChAT-positive structures corresponded well with previously proposed cholinergic elements. Control specimens showed no specific staining.

Studies of regions where cholinergic systems are less completely characterized revealed ChAT-positive somata in the caudate, parts of the globus pallidus and substantia innominata, and the cerebral cortex. Regions containing ChAT-positive, presumptive synaptic terminals included the caudate, thalamus, cerebral cortex and amygdala.

The results of the present study suggest that ChAT is present within the dendrites and axons as well as the somata and axon terminals of many cholinergic neurons. Moreover, ChAT-positive fibers and terminals appear to be organized differently in different regions of the CNS. In the spinal cord and hippocampus, they form patterned relationships either with distinct cell types or laminae. In other regions, such as the cerebral cortex and thalamus, they exhibit a more diffuse distribution, forming a loose network of fine, varicose fibers throughout the neuropil. Supported by USPHS Grant NS12116.

188.2 Immunocytochemical localization of choline acetyltransferase in the rat brain. D. M. Armstrong, C. B. Saper, A. I. Levey, B. H. Wainer, R. D. Terry*; (DMA & RDT) Dept. Pathology, Albert Einstein College of Medicine, Bronx, NY 10461. (AIL & BHW) Dept. Pathology, Univ. of Chicago, Chicago, Il. (CBS) Dept. Neurology, Washington Univ. Sch. Med., St. Louis, Mo.

The immunocytochemical localization of choline acetyltransferase (ChAT), used in the biosynthesis of acetylcholine, was employed to map the neuroanatomical locations of cholinergic neurons in rat brain. Antiserum was localized by the peroxidase-antiperoxidase method in 50um Vibratome sections of rat brain fixed by vascular perfusion with a mixture of cold 4% paraformaldehyde and 0.1% glutaraldehyde.

Four major cholinergic cell groups were found: (1) ChAT-labelled neurons were observed scattered throughout the neostriatum (caudate, putamen) and associated areas (nucleus accumbens, olfactory tubercle). (2) Larger ChAT-labelled neurons were seen in an extensive cell system corresponding to the magnocellular basal nucleus. This more or less continuous set of neuronal clusters included neurons of the medial septal nucleus, nucleus of the diagonal band (both horizontal and vertical limbs), magnocellular preoptic nucleus and basal nucleus of Meynert. We and others have found that these cell groups provide extensive topographically arranged projections to cerebral cortex. (3) A group of large neurons showing ChAT-immunoreactivity was seen in the caudal midbrain, extending dorsally and medially from the caudolateral edge of the substantia nigra and enveloping the superior cerebellar peduncle. This cell group was coextensive with the descending projections of the substantia nigra, and included the pedunculopontine tegmental nucleus (PPT) up to its imperceptible merging with parabrachial nucleus, as well as a population of neurons in the lateral part of the central gray matter. We have recently observed projections to many forebrain extrapyramidal structures from PPT, and direct cortical projections from PPT and the lateral central gray matter. (4) Both visceral and somatic cranial nerve neurons contained ChAT-immunoreactivity.

The results of the present study were largely consistent with earlier observations of cholinergic systems, thus confirming the specificity of the antibody. In addition, failure to detect ChAT immunoreactivity within intrinsic cortical neurons suggests that virtually all of the cortical cholinergic projections arise in the magnocellular basal nucleus and the more caudal cholinergic cell groups (PPT, lateral central gray, parabrachial nucleus) from which there are known projections to the cerebral cortex.

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188.3 ULTRASTRUCTURAL OBSERVATIONS OF THE LARGE,ACETYLCHOLINESTERASE-RICH NEURON IN THE RAT STRIATUM AFTER DIISOPROPYLFLUOROPHOSPHATE TREATMENT. K. Satoh*, S. Atmadja*, W.A. Staines* and H.C. Fibiger. Div. Neurological Sciences, Univ. of British Columbia, Vancouver, B.C., V6T 1W5.

It has been proposed that the large, aspiny neurons in the striatum are cholinergic (Fibiger, Brain Res. Rev., in press). In the present study, the fine structure, including synaptic relations, of these neurons in the rat striatum was investigated. Cholinergic neurons were identified by a combination of pharmaco-histochemistry for acetylcholinesterase (AChE) and electron microscopy. Animals were injected intramuscularly with diisopropylfluorophosphate (2.0 mg/kg) 2-8 hours before perfusion with aldehydes. Vibratome sections (50-60 μm) were processed for AChE cytochemistry (Karnovsky, J. Cell Biol., 23, 1964). With light microscopic assessments, small pieces of Epon sections containing intensely stained perikarya were serially cut on an ultratome. Electron microscopic examination of the cell soma and the proximal part of its dendrites was performed by employing more than 100 serial ultrathin sections with accompanying semithin sections (0.5-1.0 μm).

Fusiform or polygonal, large cell bodies (20-30 μm) containing numerous electron dense reactive particles within the cytoplasm showed ultrastructural features that have been noted previously as being characteristic of 'giant cells' (Kemp & Powell, Phil. Trans. R. Soc. Lond. 262, 1971). They were characterized by the indented nucleus with eccentric position and also by rich cytoplasmic organelles. Reactive particles were concentrated densely in stacks of rough endoplasmic reticulum which formed a typical Nissl body in the periphery of the perikaryon. The proximal dendrite (1-2 μm in diameter) with fine reactive deposits also contained abundant cytoplasmic structures; i.e. parallelly arranged microtubules, elongated mitochondria, numerous ribosomes and rough endoplasmic reticulum. Only rarely were small protuberances observed in such dendrites.

Proximal dendrites of AChE-rich cells received many synaptic contacts with symmetric membrane thickenings. The presynaptic terminals contained small vesicles, about 45 nm in diameter. The number of vesicles varied remarkably in the different terminals. Relatively large sized terminals (1-2 μm in diameter) were filled with numerous vesicles, while smaller types (<1 μm) contained a small number of vesicles, which were mostly found near the contact membrane, as well as other cytoplasmic organelles, thus giving the terminal a paler appearance. In as few instances, large vesicles were found in the cytoplasm of presynaptic terminals.

188.4 BIOTINYLATED PROTEIN A: A SENSITIVE AND VERSATILE REAGENT FOR IMMUNOCYTOCHEMISTRY. G. Nilaver, N. Latov*, S. Ginsburg*, and E. A. Zimmerman, Depts. of Neurology and Rehabilitation Medicine, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

The indirect immunoperoxidase bridge technique of Sternberger (Immunocytochemistry, 1975) is commonly used for the localization of intracellular and cell surface antigens, as well as receptors. A major limitation of this technique is that the peroxidase-antiperoxidase (PAP) complexes have to be derived from the same species as the primary antibody (i.e. rabbit PAP for rabbit antibodies). Protein A (PA) derived from Staphylococcus aureus reacts with immunoglobulin of several mammalian species, and has consequently been used in a modified PAP technique for the detection of immune complexes with primary antibodies derived from a wide variety of species (Notani et al., J. Histochem. Cytochem., 1979). Based on high affinity avidin-biotin interactions, biotin labeled second antibodies and enzymes have recently been used to form avidin-biotin complexes (ABC), resulting in highly sensitive enzyme immunohistochemical staining procedures that yield superior results when compared to the unlabeled antibody (PAP) method (Hsu et al., J. Histochem. Cytochem., 1981). In this study biotin-labeled protein A (B-PA) was substituted for the second antibody in the ABC technique, and the procedure evaluated for the immunocytochemical localization of a variety of tissue antigens, employing primary antibodies generated in different mammalian species. Lyophilized PA was biotinylated employing biotinyl-N-hydroxysuccinimide, prepared as previously described (Bayer et al., Methods Enzymol., 1979). Primary antisera generated in rabbit (to AVP), guinea pig (to insulin) and sheep (to tyrosine hydroxylase) were tested on paraffin embedded sections of rat brain, pancreas and adrenals respectively, employing the B-PA in the ABC technique. Results of staining were compared to those obtained with the conventional PAP technique as well as with peroxidase conjugated protein A. With rabbit and guinea pig primary antisera, the use of B-PA in the ABC technique resulted in high staining intensity and negligible background, with dilutions (primary antiserum) 10 to 20 fold excess of that used in the PAP technique. Antiserum raised in sheep was less reactive with PA, requiring a secondary incubation with rabbit anti-sheep IgG prior to the application of B-PA.

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188.5 **^{3}H-GLYCINE-UPTAKE NEURONS IN LAMPREY SPINAL CORD.** P.H. Sheridan*, L.J. Youngs*, N.R. Krieger and M.E. Selzer (SPON: *R.P. Lisak*). *Department of Neurology, University of Pennsylvania* School of Medicine, Philadelphia, PA 19104.

As part of a study of the synaptic transmitters used by identified neurons in the lamprey central nervous system, we have mapped the neuronal uptake of ^{3}H-glycine in the spinal cords of large larval sea lampreys Petromyzon marinus. Spinal cords were removed from the animals under Tricaine anesthesia and incubated in 10^{-6}M ^{3}H-glycine for 15 minutes. They were rinsed 6 times for 5 minutes each in lamprey solution, fixed in phosphate-buffered 2% glutaraldehyde for 2 hours, and washed in phosphate buffer. They were then sectioned with a cryostat at 16μ or dehydrated, embedded in Epon, and sectioned at 1-4μ. Sections were coated with a photographic emulsion and incubated at 4° C for 1-7 days.

By sectioning horizontally, it was possible to obtain complete serial sections of up to 1.5 mm lengths of cord in 100-150 sections. The outlines of labelled cells were traced with a Nikon drawing attachment. For one Epon embedded spinal cord sectioned at 3μ, tracings were superimposed to form complete maps for 0.6-1.5 mm lengths in three representative regions of cord: rostral (gill region), caudal (dorsal fin region), and midsection. Labelled neurons were distributed throughout the central gray columns. The cells were small (5-10μ) and numbered about 24 per hemisegment. No significant differences were observed among the three regions. A similar distribution of glycine-uptake neurons was seen in recently transformed adult sea lampreys.

None of the previously identified cell types were labelled, including lateral interneurons, edge cells, giant interneurons, dorsal cells, and Müller and Mauthner axons.

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188.6 GLUTAMINASE-LIKE IMMUNOREACTIVITY IN THE HIPPOCAMPUS OF THE RAT AND GUINEA PIG, R.A. Altschuler, R.J. Wenthold, W.G. Haser*, *D.T. Monaghan, C.W. Cotman, and J. Fex*. *Lab. Neuro-otolaryngology*, N.I.H., Bethesda, MD 20205, Dept. Biochem., Univ. Pittsburg, Pittsburg, PA and Dept. Pyschobiology, Univ. of California, Irvine, CA 92717.

Studies on the auditory nerve have led us to believe that glutaminase might serve as a marker for neurons and terminals where aspartate/glutamate is used as a neurotransmitter. In this study immunocytochemical techniques with antisera raised in rabbits against phosphate dependent glutaminase from rat kidney, were used to determine the localization of glutaminase in the hippocampus of the guinea pig and rat.

The indirect immunofluorescence technique of Coons was used on cryostat sections for LM visualization and the PAP technique of Sternberger was used on free-floating vibratome sections for LM and EM visualizations. A series of rats with kainic acid or colchicine injected unilaterally into the hippocampus, or with entorhinal lesions, were also examined with immunofluorescence techniques.

In both the rat and guinea pig glutaminase-like immunoreactivity could be seen in stratum lucidum of the regio inferior. At the EM level these could be identified as mossy fiber endings. In rats receiving colchicine injections 14 days prior to perfusion the number of granule cells in the dentate gyrus was severely reduced and no immunofluorescence was seen in the stratum lucidum. Neither kainic acid injections nor entorhinal lesions significantly affected the immunoreactivity in stratum lucidum.

It has previously been suggested that the hippocampal mossy fiber pathway might use an excitatory amino acid as a transmitter. The present study lends support to aspartate or glutamate as the transmitter of this pathway.

188.7 GABATRANSAMINASE-CONTAINING NEURONS IN RAT BRAIN. T. Nagai*, P. L. McGeer and E. G. McGeer. Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Most previous histochemical methods for GABA-transaminase (GABA-T) have failed to yield detailed information on neuronal distribution of the enzyme because of high general levels and glial localization. Previous data from our laboratory demonstrated that the technique of staining for GABA-T following suppression with an irreversible GABA-T inhibitor, such as ethanolamine-O-sulfate, will provide significant information with respect to GABA-T containing cell bodies and fibers.[1] The technique is analogous to that used for AChE localization following DFP administration which primarily reveals regenerated enzyme. Gabaculine is another irreversible GABA-T inhibitor which has the advantage over ethanolamine-O-sulfate that it crosses the blood/brain barrier. Gabaculine was administered intravenously to rats which were then sacrificed by perfusion with fixative 12 to 48 hr later. Fixed tissues were stained for GABA-T by the previously described method.[1] At a suitable post injection survival time, intense GABA-T reactivity was found in numerous cell groups. For example, positive somata and apical dendrites of cerebellar Purkinje cells were identified. In the striatum, intensely positive cells were found throughout the nucleus. Larger size intensely positive cells were observed in the globus pallidus and the substantia innominata. In the substantia nigra, many intensely positive cells were found in both the zona reticulata and pars lateralis. Positive cells were found also in the nucleus septalis lateralis, nucleus mamillaries lateralis and nucleus posterior hypothalami. Numerous small size, intensely positive cells were found in the cerebral cortex just above the corpus callosum, i.e. in the deepest layer.

[1]Vincent, S.R., Kimura, H. and McGeer, E.G., Neurosci. Lett. 16:345-348, 1980.

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188.8 QUANTITATIVE HISTOCHEMICAL MEASUREMENT OF GABA-TRANSAMINASE: METHOD FOR EVALUATION OF INTRACEREBRAL LESIONS PRODUCED BY EXCITOTOXIC AGENTS. Cornelia Sarvey*, John Stork*, Judith A. Childs*, Barbara L. Yalisove*, Ruth E. Dayhoff* and Karen Gale, Dept. of Pharmacology, and Medical Computing and Biophysics Div, Georgetown Univ. Sch. of Med. & Dent. and VAMC, Washington, DC.

GABA-transaminase (GABA-T) activity of serial fresh frozen coronal sections through rat caudate-putamen (CP) was evaluated 4-6 weeks after intrastriatal application of kainic or ibotenic acid. Thin sections (16u) were processed for GABA-T histochemistry using the method of van Gelder (J.Neurochem.12:231,'65) with minor modifications. Alternate thick sections (200u) were assayed for GABA-T activity in vitro (deBoer and Bruinvels,J.Neurochem.28:471,'77). Sections stained for GABA-T were evaluated quantitatively by computerized densitometry using image analysis.

Gross examination of CP sections stained for GABA-T revealed an obvious lack of staining in the vicinity of lesions produced by either kainate (0.5-1.0ug) or ibotenate (10-20 ug); the extent of each lesion was clearly delineated by the stain. Quantitative analysis of the stained sections revealed that the lesioned tissue contained 80-90% less GABA-T activity than control CP tissue. This loss of GABA-T was in agreement with values obtained in adjacent sections assayed in vitro for GABA-T activity. In contrast to sections stained for GABA-T, gross examination of Nissl-stained sections did not reveal obvious differences between lesioned and non-lesioned tissue. Microscopic examination of Nissl-stained sections verified the loss of neuronal perikarya in the lesioned tissue; glial cells accounted for most of the staining in these sections.

Similar studies performed in substantia nigra demonstrated that the extent of damage induced by ibotenic or kainic acid in this nucleus and the relative destruction of tissue in the overlying reticular formation could be clearly and quantitatively evaluated using the densitometric analysis of the histochemical reaction for GABA-T. Moreover, two compartments of GABA-T were readily discriminated in substantia nigra: one associated with neural perikarya, which was sensitive to kainic and ibotenic acids (70-80% of total GABA-T), and a second associated with afferent terminals arising from forebrain projections (20-30%).

Our results suggest that following destruction of neurons with kainic or ibotenic acid, GABA-T activity is largely eliminated. Under these conditions, it appears that glial cells contribute relatively little to the GABA-T activity measured either histochemically or by direct chemical assay in homogenates. Computerized densitometry of brain sections stained for GABA-T activity is therefore a useful technique for defining kainic or ibotenic acid lesions both qualitatively and quantitatively.

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188.9 THE DISTRIBUTION OF SEROTONIN IMMUNOREACTIVITY IN CELL BODIES AND TERMINALS IN THE BRAINSTEM OF THE STINGRAY, DASYATIS SABINA. C.A. Livingston, T.C. Ritchie and R.B. Leonard. Marine Biomedical Institute and the Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

The distribution of serotonin-like immunoreactivity within the brainstem of the stingray, an elasmobranch fish, was determined using the unlabeled antibody-PAP technique. With this method, we observed a wide distribution of serotonergic cell bodies, fibers and terminals throughout the rhombencephalon and mesencephalon. Many of the stained cell bodies were clustered ventromedially within the raphe, but there was considerable spread along the ventral and lateral aspects of the brainstem. Many of these cells were closely opposed to the pial surface. At the spinomedullary junction the distribution of these cells resembled that seen in the stingray spinal cord, where cells were found ventral to the central canal along the midline and in the adjacent white matter. Additional cells were scattered in the ventrolateral reticular formation and within the rostral continuation of the lateral funiculus in the caudal medulla. More rostrally, but still in the caudal medulla, the ventral medial group increased to form a distinct cluster, and cells were observed in greater density laterally, forming wings extending along the lateral margins of the medulla. At levels that should correspond to the rostral medulla, the most dorsally placed cells of the lateral group appeared to form a distinct nucleus. A large area of the medial reticular formation contained labeled fibers, and a dense network of stained fibers was seen within the visceral sensory nuclei. At levels where the caudal rootlets of the vestibular and lateral line nerves enter the brainstem (pontomedullary junction) the number of cells in the ventromedial group decreased. Further rostrally, the ventromedial cells again increased in number and spread laterally along the ventral border of the brainstem. Approaching the isthmus, large numbers of labeled cells extended a considerable distance dorsally along the lateral borders of the brainstem. At these levels scattered serotonergic cells were also observed in the dorsomedial reticular formation. The raphe cells and the cells in the medial portions of the lateral extensions were embedded within a densely stained neuropil. Another dense accumulation of fiber staining and a few cells were seen at the base of the cerebellar peduncle. In the midbrain, immunoreactive cells were densely distributed in a band across the ventral tegmentum. Caudally the band extended along the lateral aspects of the brainstem. The rostral mesencephalon, unlike more caudal brainstem regions, contained large areas devoid of immunoreactive structures. It is noteworthy that few cells were seen in the periaqueductal gray of stingrays.

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188.10 CELLS OF ORIGIN OF THE DESCENDING AND INTRINSIC SEROTONERGIC NEURONAL ELEMENTS IN THE SPINAL CORD OF THE STINGRAY, DASYATIS SABINA. T.C. Ritchie, B.J. Williams and R.B. Leonard. Marine Biomedical Institute and Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas.

The origin and distribution of serotonergic fibers and terminals in the spinal cord of the stingray were studied with the unlabeled antibody, PAP technique and a double labeling method which allows simultaneous visualization of retrogradely transported HRP and 5HT immunocytochemical staining (PAP) within single cells. Serotonergic fibers and terminals were distributed throughout the spinal gray matter in fine varicose fibers and granules probably representing terminals. Stained neuronal elements were most dense in the superficial aspect of the substantia gelatinosa, particularly in its lateral aspect, with the density decreasing ventrally. The remaining regions of the spinal gray matter contained a uniform reticulum of varicose fibers and terminals. Serotonergic fibers were present throughout the lateral and ventral funiculi with the exception of the medial ventral funiculus. The staining was most dense in the lateral funiculus. In addition, small serotonergic cell bodies were seen on the majority of sections, despite the lack of pretreatment. These cells were restricted to the region ventral to the central canal, near the midline and more laterally within the anterior funiculus. Occasionally cells were seen at the ventromedial aspect of the ventral horn. Four to six weeks following transection at midspinal levels staining appeared normal rostrally, while below the lesion the majority of serotonergic neuronal elements disappeared. Only rarely were fibers seen in the dorsal horn, while more fibers were present in the ventral horn. Occasionally, relatively large varicose structures are observed cupping the ventral aspect of the ventral horn. The origin of this residual staining is most likely the numerous intrinsic serotonergic spinal cells. To determine the origin of the large descending serotonergic projection, HRP injections were made at midlevels in the spinal cord, which has no distinct enlargements, and the tissue sections were processed with the double labeling technique for HRP and 5HT. In the medulla, large numbers of neurons exhibiting positive staining for both 5HT and HRP were observed in areas known to contain 5HT cells in the stingray. Doubly labeled cells were clustered ventromedially in the medullary raphe complex, and spread laterally along the margin of the brainstem. Only rarely were doubly labeled neurons observed in the pons and at the ventrolateral aspect of the caudal midbrain. The distribution of spinally projecting serotonergic neurons in the stingray resembles the pattern observed in rat.

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188.11 IMMUNOHISTOCHEMICAL LOCALIZATION OF NEUROPEPTIDES IN THE RABBIT INFERIOR MESENTERIC GANGLION. H. K. Kulmala, M. A. Simmons, N. J. Dun and S. A. Lorens. Depts. of Neurol. & Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153.

We have used a modified indirect immunofluorescence technique to visualize the presence of several neuropeptides in the inferior mesenteric ganglion (IMG) of the rabbit. The modification involves treatment of ganglion sections with the elution technique of Tramu et al. (J.Histochem.Cytochem.,1978) to reduce the high degree of background staining observed in the rabbit IMG. In the rabbit brain, however, we were able to successfully demonstrate serotonin immunoreactive neurons without this modification. Since we use commercially available antibodies raised in rabbits, an anti-rabbit second antibody is required, in this case goat anti-rabbit γ-globulin conjugated to fluorescein isothiocyanate (GAR). The GAR apparently binds to rabbit γ-globulins present in the periphery, but not in the CNS, resulting in non-specific staining.

Male rabbits were perfused transcardially with 0.5 L Ca^{++}-free Tyrode's solution followed by 1.0 L 4% paraformaldehyde (PF) in phosphate buffer (pH 6.5) and, finally, 3.0 L 4% PF in borate buffer (pH 11.0). The ganglion was removed, post-fixed for 90 min, placed in 5% sucrose in Sorenson's buffer and refrigerated for at least 12 h. The ganglion was frozen by liquid nitrogen vapors and sectioned at 16 μm in a -20°C cryostat. Sections were thaw-mounted, warmed to room temperature, then rehydrated with phosphate buffered saline (PBS). Sections were dipped in a solution containing 1 V 2.5% $KMnO_4$ + 1 V 5% H_2SO_4 in 50-100 V H_2O, then incubated overnight at 4°C with primary antiserum diluted 1:50 or 1:100 in PBS containing 0.3% Triton X-100. Sections were washed with PBS, incubated at 37°C for 30 min with GAR, rewashed in PBS, coverslipped with PBS:glycerin (1:3 v/v) and examined under a fluorescence microscope. Appropriate controls were performed.

Microscopic examination revealed the presence of enkephalin, somatostatin, substance P and vasoactive intestinal polypeptide immunoreactive fibers within the rabbit IMG. None of these substances appeared to be localized selectively to any portion of the IMG. In each case beaded fibers were seen throughout the ganglion and frequently appeared surrounding the principal ganglionic neurons. No cholecystokinin, luteinizing hormone-releasing hormone or serotonin immunoreactivity was seen. Thus this modified technique allows for the use of rabbit antisera for immunohistochemistry in rabbit peripheral tissues.

We have shown elsewhere that repetitive preganglionic stimulation elicits a slow depolarization and a long lasting hyperpolarization in the rabbit IMG that are resistant to cholinergic antagonists. These membrane potential changes may be mediated by one of the above neuropeptides.

188.12 IMMUNOLOGICAL LOCATION OF THE PEPTIDE PROCTOLIN AND SEROTONIN IN IDENTIFIABLE NEURONS OF COCKROACH AND CRAYFISH. C.A. Bishop, M. O'Shea and J.J. Wine. Dept. of Pharm. Physiol. Sci., Univ. of Chicago, Chicago, IL 60637 and Dept. of Psych., Stanford Univ., CA 94305.

Uniquely identifiable neurons, which are found in the central nervous systems of some invertebrates, may provide tools for the cellular study of peptide and amine transmitter functions. Whole-mount immunocytochemical techniques have assisted our search for such cells by providing us with maps of cell bodies which may contain specific neural peptides or biogenic amines. For example, we have described a map of the cockroach CNS for the bioactive pentapeptide proctolin. Information from this map was combined with biochemical, anatomical and physiological analyses to identify individual proctolin-containing neurons (Bishop & O'Shea, 1982, J. Comp. Neurol., in press; O'Shea & Bishop, 1982, J. Neurosci., in press). We will show that one of these identified neurons is the slow depressor motoneuron which innervates the main leg depressor muscles and that this neuron and its targets may now be used to study the transmitter function of proctolin.

We have also generated maps of serotonin immunoreactivity in the cockroach and crayfish and a proctolin map for the crayfish. In cockroach, serotonin immunoreactive cell bodies were found in all ganglia of the CNS. Stained varicosities were detected in all ganglia and stained fibers in all connectives. No stained processes, however, were detected in any of the nerve roots of the thoracic or abdominal ganglia. A comparison of the proctolin and serotonin maps shows more proctolin immunoreactive cell bodies in the ventral nerve cord but more serotonin cell bodies in the brain. The proctolin and serotonin systems appear to overlap only in the sub-oesophageal ganglion and the tritocerebral lobes of the brain where they may co-occur in neurons.

In crayfish, we are investigating two large (~70 μm) ventral, bilaterally symmetric cells in the first abdominal ganglion which stain with the serotonin antibody; and two pairs of bilaterally symmetric cells in the anterior portion of each abdominal ganglion which stain with the proctolin antibody. These latter cells seem to correspond to cells that appear opaque and stain with neutral red when the crayfish is between molts (R. Roth, pers. commun.). (Supported by NIH grants NS-06684 and NS-16298.)

188.13 THE INTERPEDUNCULAR NUCLEUS OF THE RAT: CYTOARCHITECTURE AND CYTOCHEMISTRY. Lisa M. Hemmendinger and Robert Y. Moore . Dept. of Neurology, SUNY at Stony Brook, New York. 11794.

Several recent immunocytochemical studies have indicated the presence of substance P, enkephalin, somatostatin, LHRH and serotonin immunoreactive neuronal perikarya and/or fibers in the interpeduncular nucleus (IPN), but have not described their precise distribution in relation to subdivisions of the nucleus. The present study was undertaken to define the cytoarchitectonic subdivisions of the adult rat IPN and to describe the distribution of chemically defined cell and fiber populations within them.

Paraffin-embedded frontal sections through the IPN were reacted using the PAP immunocytochemical method with an antibody to cathepsin D (Whitaker et al., Br. Res. 216:109, 1981), which enhances cytoarchitectural differences. This material permits a more extensive subdivision of the IPN than previously published, particularly when compared with Nissl-stained material. Four paired and 4 unpaired subnuclei can be distinguished in the IPN on the basis of neuron size, shape, staining characteristics and packing density. The rostral half of the IPN contains the rostral dorsal (RD), rostral ventral (RV) and paired rostral lateral (RL) subnuclei. The caudal half of the IPN contains the caudal dorsal (CD), caudal ventral (CV) and 3 paired subnuclei, the dorsolateral (DL), caudal intermediate (CI) and caudal lateral (CL).

These subnuclei were examined light microscopically for the presence of specific fiber and cell populations by means of immunocytochemistry, catecholamine histofluorescence and acetylcholinesterase staining. The results of these studies are summarized in the table below.

	RD	RV	RL	DL	CD	CV	CI	CL
Substance P	+•	+	–	–	–	–	–	+
Leu-Enkephalin	+•	+	+	+	+	+	–	–
Somatostatin	+	+	+	+	+	+	+•	+
LHRH	+	+	+	+	–	+	+	+
Serotonin	+	+	+	+	+•	+	+	+
Catecholamine	+	+	–	+	+	+	+	–
Acetylcholinesterase	+	+	–	+	+	–	–	+

+ fiber staining, •cell body staining, – no specific staining

The extent of innervation of each subnucleus varies, but the localization of specific fiber and cell populations within the IPN is well-delineated and conforms to the nuclear subdivisions defined using the Nissl- and cathepsin-stained material. In addition, at least 4 sets of immunocytochemically distinct neurons are present in varying numbers in specific subnuclei in the IPN.

These studies point out the cytoarchitectonic and cytochemical complexity of the IPN. Further morphological studies are underway to extend our understanding of the organization of this nucleus.

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189.1 POSTNATAL DEVELOPMENT OF RETINOGENICULATE BOUTONS IN MONKEYS. Gay Holstein*, Tauba Pasik and Pedro Pasik. Department of Neurology, Mount Sinai Sch. Med., CUNY, N.Y., N.Y. 10029.

Profiles of retinal endings in magnocellular lamina 1 (L1) and parvocellular lamina 6 (L6) of the dorsal lateral geniculate nucleus (LGNd) were measured in coded electron micrographs of newborn, 1, 2, 4, 8 and 16 week old monkeys (M. mulatta). Using a computer assisted Coupland stereological procedure, the distribution of bouton diameters was reconstructed from areal measurements of profiles identified in at least 1200 μm^2 of net neuropil from each age-lamina condition, representing 1600-3700 μm^2 of actual tissue. This distribution was used to calculate mean bouton diameter and bouton density. The latter value was adjusted to the growth of the entire nucleus (Gottlieb et al., Soc. Neurosc., 1981) resulting in the absolute number of retinal endings per laminar type. It was found that the size distribution was essentially symmetrical in all ages sampled for both laminae. Evidence of bimodality was observed in L1 of the younger specimens. The mean diameter at birth was 1.51 µm and 1.34 µm for L1 and L6, respectively, and reached 1.78 µm and 1.57 µm at 16 weeks. Although values for L1 were typically higher, and fluctuations were apparent during development, no significant differences were found between or across laminae. Retinal bouton density was calculated using the Abercrombie correction factor for section thickness. The values at birth were 19.2 x $10^6/mm^3$ and 29.2 x $10^6/mm^3$ for L1 and L6 respectively, decreasing to 12.5 x $10^6/mm^3$ and 13.3 x $10^6/mm^3$ at 16 weeks. The density in L6 was consistently higher than that in L1. The absolute number of boutons was given by the product of laminar volume and bouton density. In L1, the total number increased from 62.4 x 10^6 to 118.2 x 10^6 during the first postnatal week, decreased by 64% of the latter value in the second week, and showed a subsequent small peak at 8 weeks. Because a pooled volume estimate was obtained for the parvocellular layers, the bouton number was calculated for the entire parvocellular LGNd based on the L6 bouton density data. The value at birth was 1031.3 x 10^6, remained stable during the first week, decreased 46% in the second week, and exhibited a second small rise at 4 weeks.

The results suggest different patterns of postnatal development in magnocellular and parvocellular retinogeniculate terminals. The changes in bouton numbers indicate a substantial initial proliferation in the former but not in the latter. Although the subsequent decrease occurs by the 2nd week in both, stabilization is evident earlier in the parvocellular layers. These findings are consistent with previous conclusions based on cell size changes occurring in early postnatal development of monkey LGNd (Gottlieb et al., Soc. Neurosc., 1980).

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189.2 THE DEVELOPMENT OF RETINOTOPIC PROJECTIONS TO THE VENTRAL LATERAL GENICULATE NUCLEUS IN THE CHICK EMBRYO. W. J. Crossland. Dept. of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201.

In neural systems that connect topographically, such as the retinotopic projection upon the lateral geniculate nucleus and optic tectum, it is not known when the topographic map is first formed. Do retinal axons grow directly to their correct topographic termination regions or do they grow widely throughout the target structure, forming tentative connections that are broken later to reform in more appropriate sites?

This problem was investigated in the ventral lateral geniculate nucleus (GLv) of the White Leghorn chick embryo during axon growth. Anterograde transport of ^3H-proline or wheat germ agglutinin conjugated to horseradish peroxidase (WGAHRP) along the developing visual pathway showed that retinal axons are adjacent to the ventral thalamic cell mass on day six of incubation. The Glv primordium separates from the ventral thalamic cell mass on day seven of incubation. Labeled retinal fibers were not found among the cells of the GLv primordium until the ninth day of incubation. Small (0.3 1) injections of a 1% aqueous solution of WGAHRP (Sigma, L-2384) were made into different portions of the retina on the ninth & tenth days of incubation. After a seven-hour post-injection survival period, the embryos were sacrificed, the brains sectioned on a freezing microtome, and the sections reacted with tetramethylbenzidine.

Observations of the reacted, counterstained sections revealed that small retinal injections produced labeling in restricted parts of the optic tectum and GLv. The regions labeled in each structure always corresponded retinotopically. Furthermore, retinal injections that labeled the growing front of axons in the caudal optic tectum did not label any region within the GLv primordium.

Thus the retinotopic map had already been established at the earliest time at which axons could be found within the GLv. Whether the map is established with the same precision found in the adult GLv remains to be investigated.

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189.3 EARLY TOPOGRAPHIC ORGANIZATION OF GENICULOCORTICAL PROJECTIONS IN HAMSTER NEONATES. J.R. Naegele* and G.E. Schneider, Dept. of Psychology and Brain Science, MIT, Cambridge, MA 02139.

The topography of the geniculo-cortical projections has been extensively studied in adult rodents. However, very little is known about the topographic order within the geniculo-cortical system during development. We examined the topographic pattern of developing geniculo-cortical projections in neonatal hamsters using the retrograde transport of horseradish peroxidase (HRP).

Injections of HRP (Sigma, VI) were made in the posterior neocortex of hamster pups on postnatal days 0 (the day of birth) through day 14. To limit the spread of HRP from the injection site, HRP (10-20%) was embedded in polyacrylamide gels (Griffin, Watkins & Mayer, 1979[1]), formed into 145 µm diam. pieces and cut into short lengths (200-300 µm). The HRP gels were dried, adhered to the tip of a glass micropipette, and introduced into the neocortex. In other experiments, HRP (5-10%) was injected by iontophoresis. Survival times ranged from 6 to 30 hours. Following perfusion, brains were sectioned at 30 or 40 µm and reacted with tetramethylbenzidine. All sections were examined with light microscopy and polarization optics. The thalamocortical projections were studied by comparing the locations of retrogradely labelled thalamic neurons following injections in different regions of the posterior cortex. Dorsal-view reconstructions of the injected hemisphere were made to show the extent and location of the injection site. Since cytoarchitecture and fiber architecture are not well developed at early ages, the region of the LGNd which contained labelled neurons was compared with the extent of the injection site in order to determine the areal boundaries of visual cortex.

On postnatal day 0 (the 16th day after conception in this species), cortical injections of HRP labelled a scattered subpopulation of cells in LGNd without apparent topographic order. By postnatal day 1, we found evidence of some topography, although the precision of the mature system was not yet evident. As early as postnatal day 5, discrete iontophoretic injections into visual cortex resulted in localized columns of labelled neurons in LGNd, the location of which varied systematically with the center of the cortical injection site. At this age, the topography of the geniculo-cortical projection corresponds qualitatively with the mature pattern seen in adult hamsters. These findings indicate that topographic order in the hamster geniculo-cortical system develops both very early and very rapidly.

[1]Griffin, Watkins & Mayer (1979), Brain Research 168:595-601.
Research supported by a Whitaker Health Sciences Predoctoral Award and Grants EY00126 and EY02621 from NIH.

189.4 IS LOCUS COERULEUS INVOLVED IN PLASTICITY OF LATERAL GENICULATE NEURONS DURING LEARNING? D.H. Cohen, C.M. Gibbs*, J. Siegelman*, P. Gamlin, and J. Broyles*. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

We previously reported that during visual conditioning cells of the avian homologue of the lateral geniculate (LGN) show modification of their light-evoked discharge. All LGN neurons respond to whole-field illumination, the conditioned stimulus (CS), and 88% respond to foot-shock, the unconditioned stimulus (US). Of the US-responsive neurons, 51% show increased discharge (Type I) and 37% decreased discharge (Type II) with median response latencies of 100 msec. Only the Type II neurons show training-induced modification. The CS-evoked discharge of neurons unresponsive to or excited by the US habituate over training. Thus, not only is CS-US convergence necessary, but a particular source of US input is required as well.

To identify this source of US input, HRP was injected into the LGN, and the distribution of retrogradely labelled neurons included the locus coeruleus, subcoeruleus, central gray, pontine reticular formation, and various non-specific thalamic nuclei. Since the mammalian literature suggests that locus coeruleus (LC) neurons respond to nociceptive stimuli, we focussed first on this source of non-visual input. Autoradiographic experiments confirmed that LC projects to the avian LGN. Moreover, neurophysiological analysis of 48 LC neurons indicated that 81% were responsive to the US, 58% showing increased and 23% decreased discharge. The median response latency was 75 msec.

For further evaluation of possible LC involvement in mediating the Type II response of LGN neurons, electrolytic lesions were made bilaterally and after 10-14 days the US responsiveness of LGN neurons was evaluated. With lesions that extensively destroyed the rostral pole of LC, 85% of LGN neurons remained responsive to the US. However, most now showed Type I responses, the Type II response being almost eliminated. This suggests the LC is the principal source of the LGN Type II response to the US. Since the proportion of unresponsive cells did not change and the proportion of Type I responses increased, the data further suggest that US responsive LGN neurons receive both Type I and Type II inputs.

After LC lesions, the Type I LGN cells had longer response latencies and decreased response magnitudes, suggesting that LC also contributes Type I input to the LGN. Supporting this are preliminary findings that electrical stimulation of LC excites some LGN cells and inhibits others. We are now investigating the possibility that these Type I and II inputs derive from different populations of LC neurons. (Supported by NSF grants BNS8016396 (DHC) and PDF8166055 (CMG) and a grant from the Sloan Foundation (DHC).)

189.5 NORMAL DEVELOPMENT IN THE C LAMINAE OF THE CAT LATERAL GENICULATE NUCLEUS. J.E. Parsons*, D.M. Murakami* and P.D. Wilson (SPON: M. Nachman). Dept. of Psychology, Univ. of Calif., Riverside, Riverside, CA 92521.

Developmental patterns of soma sizes have been examined in layers A, A1, the magnocellular and parvocellular parts of C, and in layers C1 and C2 of the cat dorsal lateral geniculate nucleus (dLGN). Four days after an intraocular horseradish peroxidase injection, cats were sacrificed and 40μm brain sections were reacted with TMB and counterstained with thionin. The anterograde transport of the HRP labeled alternate layers in the dLGN and permitted identification of the boundaries of adjacent layers.

Mean soma diameters were obtained from the middle third of the binocular portion of the dLGN in cats ranging from 2 to 16 weeks of age. Our present results are in agreement with a previous report (Hickey, J. Comp. Neurol., 189:467, 1980) that cell bodies in the A laminae continued to grow up to the 4th week. Mean soma diameters for 4 week old cats (A 15.2μm) were about 2-3μm larger than somas of 2 week postnatal cats (A 12.6μm). The magnocellular and parvocellular parts of layer C were measured separately since it has been suggested that these are two functionally different sublayers. As was the case for A and A1, both C-magno and C-parvo increased in size from the 2nd week (C-magno 12.9μm, C-parvo 11.2μm) to the 4th week (C-magno 15.7μm, C-parvo 14.9μm). The mean soma diameters in the C1 and C2 layers of 4 week old cats (C1 14.5μm, C2 14.0μm) were also substantially larger than cell sizes in the C1 and C2 layers of 2 week old cats (C1 11.6μm, C2 12.0μm). Soma size measurements in the C layers of 16 week old cats do not appear to be markedly different from those obtained in 4 week old cats. In general, we have found that for 2 to 16 week old cats the period of greatest growth for somas in all of the C laminae occurs between 2 and 4 weeks of age. (Supported in part by Biomedical Research Support Grant and Academic Senate Committee on Research, University of California, Riverside)

189.6 EFFECT OF EARLY AND LATE MONOCULAR DEPRIVATION ON CELL SIZE IN THE C LAMINAE OF THE CAT dLGN. D. M. Murakami*, P. D. Wilson, and C. A. Yarosh*. Psychology Department, University of California, Riverside, CA 92521.

We previously reported that monocular deprivation affects the cell size of neurons in layer C1 of the cat dLGN (Soc. Neurosci. 6: 789, 1980). The changes were not significant until monocularly deprived cats were at least 20 weeks old. In order to test whether the period of peak sensitivity to monocular deprivation is different for the A and C layers, cats were monocularly deprived at 12 weeks of age (LMD) and compared to cats deprived at 2-3 weeks of age (MD). The LMD cats were sacrificed for histological study at 34 weeks of age and the MD cats were examined at 16 to 22 weeks of age. In order to reveal the C laminae, 20 ul of a 30% HRP solution was injected into the vitreous chamber of one eye. After 4 days survival, the animal was sacrificed and 40 um brain sections were reacted using TMB and counterstained with thionin. Mean soma diameters were measured for at least 100 neurons from the monocular segment and from the middle third of the binocular portion of layers A, A1, and C1. Samples of at least 50 cells were taken from the magnocellular and parvocellular portions of layer C and from layer C2.

In the LMD cats, the nondeprived and deprived monocular segments had only 2.2% difference in cell size. While a larger difference was found between the nondeprived and deprived binocular portions of layers A, A1, C-magno, and C1 in the LMD cats, the differences were much smaller than in the MD cats (layer A- MD 15.2%, LMD 6.5%; layer A1- MD 16.2%, LMD 6.5%; layer C magno- MD 11.5%, LMD 6.1%; layer C1- MD 9.7%, LMD 5.8%). The effect of LMD in the binocular portion of layers C-parvo and C2 was not significantly different than the effect in the monocular segment.

The results indicate that binocular competition still affects cell size in the dLGN of cats monocularly **deprived from 12 weeks of age. However, the effect is much smaller for layers A, A1, and C-magno in LMD** cats than in cats deprived from 2 and 3 weeks of age. The effect of deprivation is also less for C1 in LMD cats than in MD cats, but the difference is less. (Supported in part by Biomedical Research Support Grant and Academic Senate Committee on Research, University of California, Riverside)

190.1 A MODEL OF VISUAL CORTICAL PLASTICITY EMPLOYING PROPRIOCEPTION FROM EXTRAOCULAR MUSCLES. M. Shadlen* and J. A. Anderson. Center for Neurosciences, Brown University, Providence, RI 02912.

We have developed a model of visual cortex which explains certain plastic properties of visual cortical (striate) neurons. We have simulated development of orientation specific striate neurons.

Development and maintenance of receptive field properties are believed to be dependent upon activity and experience of an animal. We utilize the notion that an organism's organization of information may be related to that organism's actions in and upon its environment. We suggest that the movements of the eye may be intimately related to modification of striate neuron receptive fields.

In the model we communicate, the strength of connection between the i-th presynaptic lateral geniculate nucleus (LGN) neuron and the j-th postsynaptic striate neuron is dependent on two parameters:

1) activity of the i-th presynaptic neuron,
2) a non-visual input to the j-th postsynaptic neuron corresponding to movement of the eye.

Thus the receptive field of any j-th striate neuron is determined by the type of visual inputs it receives (here spots of contrast via LGN afferents) and the particular motion with which it is associated (here by virtue of proximity to extraocular afferent terminals in the visual cortex).

Although complex patterns serve as stimuli, we simulate development of line orientation receptive fields. The model predicts the observed clock-like anatomical arrangement of such receptive fields in the visual cortex.

A number of recent reports suggest that proprioception from extraocular muscles is important in determining cortical ocular dominance (for a critical review see Movshon, J.A. and Van Sluyters, R.C., Ann. Rev. Psychol. 32:523-74, 1981). Our model indicates that if proprioceptive signals play a role in visual cortical plasticity, it is possible that they contribute to the development and maintenance of striate receptive fields. Furthermore, they might provide a mechanism whereby simple patterns such as lines may be distinguished from a complex visual environment.

190.2 INTRAOCULAR TETRODOTOXIN HINDERS GOLDFISH OPTIC NERVE REGENERATION. D.L. Edwards and B. Grafstein. Physiology Dept., Cornell University Medical College, New York, N.Y. 10021

The goldfish visual system provides a convenient model in which to study axonal regeneration since the retinal ganglion cell (RGC) responds vigorously to injury and the time course of regeneration has been well characterized. In the present study we have examined the effect of intraocular tetrodotoxin (TTX) on the time course of recovery from optic nerve crushes.

In each goldfish, one optic nerve was crushed and the corresponding eye was injected with 0.07 µg TTX in citrate buffer, a dose that blocks activity for 2.5-3 d. TTX injections were repeated every 3 or 4 d. Control fish were similarly injected with citrate buffer vehicle. The time course of optic nerve regeneration was evaluated by a) behavioral testing of recovery of visual function, b) measurements of the time of arrival of regenerating axons at the optic tectum, c) axon counts in regenerating optic nerves and d) measurements of RGC numbers, size, and level of protein synthesis.

The behavioral testing showed that recovery of the startle reaction to a bright light (mean normal recovery time = 12 d post-crush) and food pellet localization (mean recovery time = 35-40 d) were both delayed in the TTX-treated fish. Intraocular TTX had no effect on the time when axons first reach the tectum (as indicated by the amount of ^{3}H-proline labeled protein axonally transported to the tectum at 15-18 d after crushing), but at 24 d post-crush the amount of labeled transported protein arriving at the tectum in TTX-treated fish was significantly less than normal. By 36 d this difference had disappeared. Silver-stained sections of nerves, examined at both 10 and 22 d post-crush, showed 20% fewer axons at a point 1 or 1.5 mm distal to the initiation of outgrowth. However, there was no effect of intraocular TTX on the number of RGCs, on the mean RGC area at 22 and 36 d post-crush, or on the incorporation of ^{3}H-proline into protein in RGCs at 36 d. In a separate experiment only a single injection of TTX was given at the time of crushing. Recovery of the startle reaction was delayed compared to citrate controls, but there was no difference in the time of recovery of food localization.

These combined behavioral and morphological observations show that RGC axons can regenerate and form viable synapses in the presence of TTX, but suggest that the TTX treatment may diminish either 1) the diameter of the regenerating axons, 2) the extent of axonal branching in the nerve or at the tectum, or 3) the rate of outgrowth of some of the population of regenerating axons. These changes are not accompanied by gross alterations in the retinal ganglion cell body response to axotomy.

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190.3 ACTIVITY SHARPENS THE MAP DURING THE REGENERATION OF THE RETINOTECTAL PROJECTION IN GOLDFISH. J.T. Schmidt and D.L. Edwards, Biology Dept., SUNY Albany, Albany, NY 12222 and Dept. Physiol., Cornell Univ. Medical College, New York, NY 10021.

In the regenerating retinotectal projection of goldfish, we have used intraocular injections of tetrodotoxin (TTX) to test whether 1) effective synaptic connections can be formed in the absence of activity, and 2) whether activity plays a role in organizing or refining the retinotopic map. At the time of optic nerve crush, 0.07 µgm of TTX in citrate buffer was injected into the eye, a dose which blocks retinal ganglion cell activity for approximately 2½ days. Successive injections afforded a continuous 27 day block without toxic effect as evidenced by the continued movements of the injected eyes. Control fish were injected on the same schedule with citrate buffer.

Analysis of the field potentials evoked by optic nerve shock showed that fibers regenerating without activity had a normal ability to form synaptic connections in the tectum. There was a tendency for the response latency at 28-32 days to be longer than controls but this difference disappeared at longer regeneration times.

The retinotectal maps of the TTX fish were normally organized but the multiunit receptive fields were grossly enlarged. In control regenerates, one to three units (arbors of retinal ganglion cells) were recorded per penetration and their combined receptive field averaged 11-12 degrees, nearly the same as for single units. In TTX fish, each penetration yielded at least 5 to 10 units whose receptive fields were clustered over a wider area averaging 27 degrees across. (A lack of effect on individual ganglion cell receptive fields was confirmed by intraretinal recordings). Many fish were recorded up to 4 months after the release from TTX block, but no further refinement of the maps occurred. Apparently activity is needed during the regeneration process. If the nerve was recrushed and allowed to regenerate a second time without TTX, normal maps were formed, ruling out any permanent changes in the retinal ganglion cells. Blocks during various portions of the regeneration process showed that activity is not critical during the process of axonal elongation (first 2 weeks) but is critical during the period of synapse formation and maturation (14-34 days). The results are discussed in terms of an activity-dependent stabilization of synapses. Neighboring retinal ganglion cells are known to fire in a statistically correlated fashion and this could help in their elimination of incorrect branches following an early period of diffuse connections. Supported by NIH Grants EY03736 (To J.T.S.), NS 09015 and EY 02696 to Bernice Grafstein.

190.4 TESTS FOR A ROLE OF ACTIVITY IN THE FORMATION OF OCULAR DOMINANCE PATCHES. V. Boss* and J.T. Schmidt (SPON: A. Messer) Dept. of Biological Sciences, SUNY at Albany, NY 12222.

Following removal of one tectum in goldfish, the optic nerve fibers from both eyes are forced to compete for synaptic space in the remaining tectum, and their terminals eventually segregate into ocular dominance patches seen anatomically. This study tested whether segregation is prevented or delayed by blocking impulse activity in both eyes using tetrodotoxin (TTX).

In 3-4" goldfish (housed at 30°C) the left tectum and forebrain were removed and the right nerve deflected toward the remaining tectum. The regenerating nerve fibers would compete with the intact projection from the normal eye for synaptic space. Beginning 18 days after surgery (before segregation has begun), both eyes were given TTX injections every second day to achieve a continuous block of retinal activity. Control fish were injected with citrate-Ringers on the same schedule.

Ocular dominance patches were measured in two ways. First, 75 days after surgery HRP and ^{3}H proline were injected into the left and right eyes, respectively, to trace their projections to the tectum. Alternate frozen sections were processed for the HRP-TMB reaction and radioautography. In control fish ocular dominance patches in the projections from both eyes could be seen as early as 53 days and always by 64 days after surgery. By 75 days patches were well defined in the normal eye's projection in most control fish. In contrast, in the TTX fish very few patches could be seen in the the projection from the normal eye at 75 days; thus the segregation of the projections from the two eyes may be delayed when activity is blocked.

Second, the tecta of some of the 75 day fish were examined electrophysiologically using the field potentials evoked by optic nerve shock. Analysis of sources and sinks of synaptic currents provided a measure of the postsynaptic response to each eye. In control fish there were local maxima and minima in the responses to each nerve. The responses to the regenerating nerve were generally larger in those areas where the normal eyes' responses were small, and vice versa. In TTX fish the responses to both nerves were more uniform across the tectum. Lesions were placed at each penetration for correlation with the anatomical patches. The physiological data, like the anatomical data, suggests that the ocular dominance patches either fail to form or their formation is delayed in the absence of neuronal activity.

Supported by NIH Grant EY 03736 to JTS.

190.5 THE ROLE OF TEMPORAL ACTIVITY DURING AUDITORY MATURATION. D. H. Sanes* and M. Constantine-Paton. Dept. of Biology, Princeton Univ., Princeton, New Jersey, 08544.

We have begun to examine the role played by the temporal patterning of neural activity during the development of auditory tuning curves. Our paradigm involved raising young mice in an environment which entrains a large proportion of auditory nerve fibers, repetitive clicks. Our expectations were based on the hypothesis that coactive neural inputs might be more likely to form lasting connections with a post-synaptic neuron. Precise convergence onto post-synaptic cells might then be effected by selecting inputs with temporally similar action potential patterns from an initially larger array of terminals. If this were correct, then in the absence of temporal identity, such as when fibers are driven synchronously, functionally inappropriate connections should result. The post-synaptic neuron would then be expected to have less specific response properties.

Frequency tuning curves were taken from single units in the central nucleus of the inferior colliculus of normal and click-reared (CR) mice, aged 18-24 days. Q values, indicative of the degree of tuning, were calculated for both groups. The CR animal tuning curves were found to be significantly broader (t-test; $p<.01$) for units with best frequencies in the range 10-14.8 KHz. The CR tuning curves in this region seemed to be generally broader from the tip region (Q5) to at least 40 dB above threshold (Q40). These results were true of animals raised at a click repetition rate of 8 pulses per second. The effect was even more pronounced for animals raised in a higher repetition rate (20/s). These showed less variability and somewhat broader tuning curves than mice raised at 8/s.

A Fast Fourier Transform of the click used in rearing revealed that the power spectrum decreased dramatically above 15 KHz. Below this point the power spectrum was virtually flat (+/-5 dB). It was also found that mouse pups produce extremely narrow-band squeaks below 10 KHz. In this region the clicks did not seem to have any effect.

We do not believe that these results can be attributed to damage or trauma in the cochlea because the mean threshold for normal and CR units were identical for all frequency ranges. Furthermore, a small percentage on units in the 10-14.8 KHz range were sharply tuned. Nevertheless, we intend to fully test this possibility through physiological analysis.

At present our data is fully consistent with the hypothesis that temporal patterns of activity are involved in ontogenetic tuning of the response properties of neurons in the central auditory pathway.

(Supported by NIH Predoctoral Training Grant 07312)

190.6 SILENCING OF NATIVE AFFERENTS PERMITS INNERVATION BY FOREIGN AFFERENTS IN A FIRST-ORDER SENSORY INTERNEURON OF CRAYFISH. F.B. Krasne, D.L. Glanzman, and J.S. Gunther. Dept. of Psychology and Brain Res. Inst., University of California, Los Angeles, L.A., CA 90024.

Effects on neuron activity on synapse formation and elimination have been shown to be important in the developing mammalian visual system and in vertebrate skeletal muscle. However, whether such effects are due to general properties of neurons or to special properties of muscle and developing visual system is presently uncertain. We report here results which encourage belief in their generality.

Interneuron A (hereafter A) of crayfish last abdominal ganglion normally receives direct input from mechanoreceptors borne on the animals ipsilateral tail fan appendages. The axons of the peripheral mechanoreceptor somata travel to the ganglion over ganglionic roots 1-5. If these roots are cut, the axons regenerate, growing back into the ganglion and reforming synapses with A, as indicated by return of A's responsiveness to water currents. Cut contralateral roots 1-5, if tied across the midline, can substitute for native ipsilateral roots; however, such crossed innervation can ordinarily only be produced if A has been denervated.

We have now found that such crossed innervation can be produced even with ipsilateral roots intact if the sensory axons of these roots are silenced by immobilizing tail fan tactile hairs with glue (Histoacryl Blue). We feel reasonably confident that the glue is not damaging the receptors and causing degeneration, because responsiveness of A to water disturbances of glued fields returns to normal when animals shed their exoskeletons (and glue) at molt and because electrical stimulation of roots whose peripheral fields are glued remains fully competent to drive A.

Supported by USPHS grant NS 08108 to F.B.K.

190.7 EFFECTS OF DIFFERENTIAL ENVIRONMENTS AND HIBERNATION ON GROUND SQUIRREL BRAIN MEASURES. M. R. Rosenzweig, E. L. Bennett, M. Alberti,* H. Morimoto* and M. Renner. Dept. of Psychology and Melvin Calvin Lab., Univ. of California, Berkeley, CA 94720.

As part of a study of brain plasticity, we investigated development of brains of Belding's ground squirrels (Spermophilus beldingi) in two phases and under two kinds of environmental conditions. Phase I involved exposure to enriched or impoverished environments. Pregnant females were trapped in the Sierra in June and brought to the laboratory where they bore their litters. Shortly after weaning, the pups were assigned to either of two conditions: (1) Enriched Condition (EC) placed in groups of 10-12 in relatively large cages that contained diverse stimulus objects. (2) Impoverished Condition (IC) -- individuals in small cages without stimulus objects. Similar experiments were conducted during three summers, 1978-80. The length of the experimental period ranged from 47 to 75 days. Each year shortly before the termination of the experiment, feral juvenile ground squirrels of about the same age were live trapped in the same location as the females of that year. At sacrifice, the brains were dissected rapidly into 6 standard samples, weighed, and stored for analysis of nucleic acids. The feral and EC squirrels were found to be similar in most brain measures, and both exceeded the ICs significantly in brain weights, total RNA and total DNA. The only way in which the feral squirrels differed consistently from the lab-raised squirrels was that they showed greater RNA/tissue weight. Phase II took place in October-May and involved placing some juveniles in individual cages in a cold room ($\sim 2.5^0$ C) while others remained in EC at normal room temperature ($\sim$22). A baseline group was sacrificed at the outset of this phase and other groups were sacrificed at various times during the winter and early spring. After a preliminary hibernation experiment in 1978-79, further experiments have been conducted in 1979-80, 1980-81, and 1981-82. In experiments through 1981, hibernators sacrificed in May showed significantly greater total RNA than either the prehibernation baseline group or the winter-awake squirrels; this difference amounted to $\sim$15% whereas the significant effects of enriched vs. impoverished environments were only $\sim$5%. This research received support from NSF Grant BNS 791374 and from the Division of General Life Sciences, Dept. of Energy Contract DE-AC03-76SF00098.

190.8 SPINAL CORD, ROOTS AND NERVES: A UNIT EXHIBITING INJURY-INDUCED ALTERATIONS? L. J. Fisher*, J. A. Beel*, L. S. Stodieck*, and M. W. Luttges (SPON: R. Gerren). Department of Aerospace Engineering Sciences, University of Colorado, Boulder, CO 80309

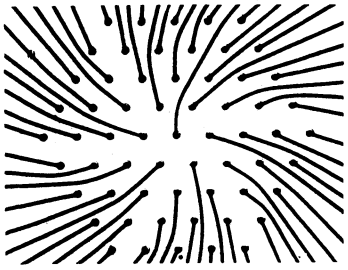
Previous studies (Luttges et al., 1976; Gerren and Luttges, 1979) demonstrated segmental alterations bilaterally as a consequence of unilateral sciatic nerve damage. These studies, combined with our current work, indicate such time-dependent segmental alterations may be functionally and structurally interrelated.

Sciatic nerves, ipsilateral and contralateral to nerve crush, were examined biomechanically. A bilateral, time-dependent increase in nerve strength and stiffness was observed. These alterations may have important consequences for attached nerve roots, terminals and spinal cord. Any absence of compensatory alterations may lead to an increased potential for mechanical injury elsewhere. These possibilities prompted an examination of injury-induced alterations in spinal cord protein constituents and metabolism using SDS polyacrylamide gel electrophoresis and radioactive amino acid precursors. Results from these studies indicate no overall change in protein composition or synthesis exhibited by the spinal cord in response to unilateral nerve crush. In contrast, alterations in synaptic terminal morphology have been reported and ongoing work reveals large differences in γ-amino butyric acid uptake and binding by synaptosome preparations obtained from the spinal cord following nerve damage. The overall structural stability of the spinal cord is the background against which specific segmental elements exhibit characteristic time-dependent alterations. This contrast also is evident in ortho- and antidromic electrophysiological studies focused on the spinal cord. Time-dependent, electrical alterations are observed bilaterally in both the spinal cord and nerves. Thus, a "segmental unit" can be defined as encompassing spinal cord, roots and nerves which share time-dependent alterations in response to experimental nerve damage. Injury-induced changes in the unit may subsequently affect other physiological functions supported by the unit. The comprehensive nature of the unitary segment must be considered when evaluating the consequences of incurred injury.

190.9 STUDIES OF CULTURED CELLS IN DISHES INCORPORATING INTEGRAL MICROCIRCUIT ELECTRODES. J. Pine and J. Gilbert*. California Institute of Technology, Pasadena, California 91125

The fact that cultured nerve and muscle cells grow as a monolayer, in close proximity to a flat substrate, makes it attractive to utilize electrodes embedded in the dish bottom to extracellularly stimulate nearby cells. It is also possible to use such electrodes to record signals arising from action potentials in nearby cells. Preliminary experiments have shown the feasibility of this technique in cultures of rat superior cervical ganglion neurons. (J.Pine, J. Neuroscience Meth., 2:19-31, 1980.)

We have fabricated culture dishes for studying small networks of nerve or muscle cells growing as microcultures in a region approximately 0.5 mm. in diameter at the center of the dish. This region lies over an array of 61 microelectrodes spaced 70 microns on centers, such that all locations in the region are within range of at least one microelectrode. The dish bottoms are glass cover slips on which a vacuum-deposited gold pattern defines electrodes about 15 microns in diameter and connecting leads radiating outward to the edge of the dish. The figure below shows the central region of a dish:



An insulating layer covers the gold pattern, with openings etched through it at the electrodes. The electrodes are platinized electrolytically to achieve a low impedance to the culture medium. The dish assembly, formed by cementing a lucite ring to the glass cover slip, is about 1 cm. square, and is mounted on a commercial integrated circuit chip carrier. External electronics can be used to record and stimulate from several electrodes simultaneously.

We are studying the effects of chronic stimulation on development of nerve and muscle cultures, and will report preliminary results.

190.10 SUPPRESSION OF ELECTRICAL ACTIVITY IS ASSOCIATED WITH NEURONAL DEFICITS: DEVELOPMENTAL ONSET AND NEUROCHEMICAL SPECIFICITY. Douglas E. Brenneman*, Elaine A. Neale, Linda M. Bowers*, and Phillip G. Nelson (SPON: Bruce K. Schrier). Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, Maryland 20205.

The role of electrical activity in neuronal survival was assessed in cultured neurons. Chronic blockade of spontaneous electrical activity with tetrodotoxin (TTX) produced neuronal deficits. The developmental vulnerability and neurochemical specificity of this effect was investigated in dissociated cultures of spinal cord and dorsal root ganglion cells. Cultures were prepared from 12-14 day old fetal mice.

The response of neurons to electrical blockade was estimated with cell counts and two neuronal surface markers: ^{125}I-tetanus toxin fixation and ^{125}I-scorpion toxin binding. Application of TTX during the 2nd or 3rd week in culture resulted in cell counts which were 68-75% of control. Both surface labels were decreased to 55-60% of control values after TTX treatment during this same developmental period. No changes from controls were observed after one week exposures of TTX during the 1st or 4th week in vitro as measured with tetanus toxin fixation.

Tetanus toxin fixation was saturable as a function of cell plating density. At high plating densities, neither an increase in initial plating density nor tetrodotoxin treatment affected the amount of tetanus toxin fixation.

Evidence of developmental sensitivity and neurochemical specificity for the neuronal deficits produced by electrical blockade was obtained with choline acetyltransferase (CAT) assays. Suppression of electrical activity during the first six days in culture had no effect on CAT activity and no effect on the appearance of the neurons. Application of TTX during the 7th day in culture decreased CAT activity to 68±4% of control. A 24 hour exposure to TTX on the 8th day in culture resulted in CAT activity only 58±4% of control. More chronic (3 weeks) treatment with TTX revealed that the decrease in CAT activity was related to the duration of electrical blockade.

Glutamic acid decarboxylase (GAD) activity was not changed after chronic application of TTX. Similarly, high affinity ^{3}H-GABA uptake was not altered by this cessation of electrical activity. ^{3}H-GABA autoradiography revealed no change in the number or appearance of GABA-positive cells after TTX treatment.

These data indicate that blockade of spontaneous activity produces a deficit in developing neurons in culture. This effect exhibits specificity both with regard to the developmental stage at which vulnerability occurs and with regard to the neurochemical system which is affected.

190.11 EFFECT OF FIBRILLATION ON AChE IN CULTURED RAT MYOTUBES S. Younkin, S. Brockman*, J. Newman*, and L. Younkin, Dept. Pharmacology, Case Western Reserve Univ., Cleveland, Ohio 44106

Fibrillating myotubes (derived from 20-day rat embryos) were compared with myotubes in which fibrillation was blocked on the fourth day of culture with 1 μM TTX. Cultures were sequentially extracted to separate globular, asymmetric, and non-extractable AChE. Individual globular and asymmetric forms were then analyzed by velocity sedimentation. At 5 days, control myotubes (which were just beginning to fibrillate) and TTX myotubes contained essentially the same level of AChE and the same distribution of the various forms of the enzyme - 71% globular (49% 4S, 22% 10S), 15% asymmetric, and 14% non-extractable AChE. From day 5 to day 8, overall levels of AChE increased 8.1-fold in fibrillating myotubes and 2.6-fold in TTX myotubes. Globular forms increased 6.4-fold (7.8-fold 4S, 3.5-fold 10S), asymmetric forms 14.9-fold, and non-extractable AChE 9.1-fold in fibrillating myotubes whereas in TTX myotubes globular forms increased 2.3-fold (2.6-fold 4S, 1.4-fold 10S), asymmetric forms 3.3-fold, and non-extractable AChE 3.3-fold. All of the major forms of AChE present in innervated adult skeletal muscle were present at 8 days in both fibrillating and TTX myotubes. Fibrillating myotubes contained 58% globular (48% 4S, 10% 10S), 27% asymmetric forms (4% 12.5S, 10% 16S, 13% other) and 15% non-extractable AChE. TTX myotubes contained 62% globular forms (51% 4S, 11% 10S), 23% asymmetric forms (4% 12.5S, 5% 16S, 14% other), and 15% non-extractable AChE. Careful light microscopic evaluation of fibrillating and TTX myotubes showed that the effect of TTX on AChE was not due to gross impairment of morphological development. Titration of AChE active sites in 8-day control and TTX myotubes with O-ethyl-S-diisopropylaminoethyl methyl-phosphonothionate showed that the reduction in AChE activity in TTX myotubes was primarily, if not exclusively, due to a reduction in the number of AChE molecules. To evaluate the effect of fibrillation on the synthesis of globular and asymmetric forms of AChE, we inactivated essentially all of the AChE in 7 day cultures of fibrillating and TTX myotubes with either methanesulfonyl fluoride or paraoxon and carefully evaluated the recovery of AChE. Our analysis indicates that fibrillation substantially increases the rate at which both globular and asymmetric forms are synthesized. We conclude 1) that muscle cells produce all forms of AChE in the absence of electromechanical activity and 2) that electromechanical activity substantially increases the level of both globular and asymmetric forms of AChE primarily, if not exclusively, by increasing synthesis. There are differences between our study and an earlier study (Rieger et al., Dev. Biol., 76: 358-365; 1980) that will be discussed.

190.12 LACTATE DEHYDROGENASE VARIATIONS AMONG SINGLE CULTURED MYOTUBES DUE TO CONTRACTION. P. M. Nemeth, L. Solanki* and J. C. Lawrence, Jr.* Depts. of Neurology and Pharmacology, Washington Univ. Med. Sch., St. Louis, MO 63110.

The effects of contraction on lactate dehydrogenase (LDH) activities of single cultured myotubes has been studied. Skeletal muscle cells were obtained from 19 day rat fetuses and allowed to fuse in vitro into spontaneously active myotubes. To obtain samples for enzymatic analysis, the cultures were rinsed with an isotonic solution of ammonium formate and freeze-dried. Individual myotubes were manually dissected from the lyophilized samples and LDH activities were measured by microanalytical biochemistry on 3-25 segments (each weighing 2-10 ng). Some variation of enzyme activity was found along the length of individual myotubes (average coefficients of variation 0.13±0.06). The LDH activity among individual cells after 1 week of culture (n=34) was 20 to 120 moles/kg/hr with an average of 70±22 (coefficient of variation 0.32). The heterogeneity among fibers was of the same magnitude as found in mature rat hindlimb muscles. Heterogeneity in vitro may be partly explained by the observed uneven contractile patterns within the culture. To test this idea, contractions were completely inhibitied by incubating the cultures with 10^{-5} M tetrodotoxin (TTX). Paralysis of 12 day old cultures increased LDH activity from 84 (12 day old controls, n=16) to 100 moles/kg/hr (n=24). Moreover, the paralysis enhanced the uniformity of enzyme levels among the cells. The coefficient of variation for LDH was 0.25 in normal compared to 0.15 in TTX-treated cultures. Enzyme variation along the length of paralysed myotubes was similar to normal cells; the average coefficient of variation was 0.12±0.05. The results show that primary cultures of rat myotubes are heterogenous with respect to LDH activities. Inhibition of spontaneous contraction reduces the differences among cultured myotubes. (Supported by MDA and by NIH AM28312 and NS18387 grants.)

190.13 EFFECTS OF ALTERED MOTOR UNIT ACTIVITY ON NORMAL AND REINNERVATING MUSCLE. K. E. Misulis* and W-D. Dettbarn (SPON: A. Burt). Neuromuscular Dis. Res. Ctr., Vanderbilt Univ., Nashville, TN 37212.

Nerve-muscle interactions such as muscle activity and neurotrophic factors are important for the development and maintenance of normal neuromuscular characteristics. One of these interactions, the patterns of electrical activity imparted to the muscle fibers by innervating nerves, was studied here.

Two models of altered motor unit activity were used to investigate the role of activity on normal and reinnervating fast and slow muscle. In the hypokinetic model, rats were suspended with the hindlimbs fully unloaded and the head tilting downward. Under pentobarbital anesthesia surgical gauze was wrapped around the base of the tail with silk tape and silastic; the attached gauze was secured to a small swivel snap clip (commercially available) which was clipped onto one bar of the top of an animal cage. This arrangement allowed locomotion around the cage by the forelimbs with rotation provided by the swivel clip and longitudinal movement by sliding of the clip on the bar. This technique permits patterned movement of the hindlimbs but no weight bearing.

The second model was the spinal rat. Under deep pentobarbital anesthesia adult rats were spinalized at T10 via a dorsal laminectomy. After a brief period of spinal shock the hindlimbs maintained a spastic posture. This model represents an increase in resting muscle tone, but the relative absence of movement-patterned phasic activity.

In both of these groups the left sciatic nerve was crushed for the study of reinnervation. The contralateral non-crushed side served as control. Parameters such as acetylcholinesterase (AChE) activity, muscle mechanics, and muscle and nerve electrical activity were studied.

Spinalization alone produced an increase in the relative activity of AChE in the soleus, but a decrease in the EDL. In contrast, hypokinesia resulted in an increase in AChE in both the soleus and EDL. Studies of reinnervation under these conditions revealed an apparent delay in recovery of mechanical activity (maximal twitch tension and rate of rise) and altered patterns of AChE recovery in spinal animals.

The results of experiments performed with these models will hopefully give insight into the role of electrical activity in neurotrophic phenomena, and in addition, indicate what factors are important in guiding and supporting reinnervation of muscles.

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190.14 CONTRACTILE PROPERTIES OF REINNERVATED RAT GASTROCNEMIUS MUSCLE : EFFECTS OF LOW FREQUENCY STIMULATION. B. G. Cole*, P. F. Gardiner and C. W. Y. Chan. School of Physical and Occupational Therapy, McGill Univ., 3654 Drummond, Montréal, Qué., H3G 1Y5 and Dep't d'Education Physique, Univ. de Montréal, Montréal, Qué. H3C 3J7

Direct low frequency stimulation of denervated muscle has been shown to reduce the atrophy which follows peripheral nerve injury. However, its influence on the contractile characteristics of fast muscle following reinnervation has yet to be established. The object of this study is to determine the effects on the in situ contractile properties of reinnervated rat gastrocnemius muscle of direct low frequency stimulation begun the day following denervation. Twenty-five adult rats were randomly divided into 3 groups - I = crush/stimulation; II = crush/no stimulation; III = control. In groups I and II, the left sciatic nerve was crushed at the level of the sciatic notch. The left gastrocnemius muscles of animals in group I were stimulated percutaneously at a frequency of 20 Hz for 5 min, 3 times daily. Maximal isometric contractions were elicited. Treatment began the day after surgery and continued until evaluation (8 wks following surgery) when muscle contractile and fatigue characteristics were assessed. Reinnervated muscles (groups I and II), whether stimulated or not, showed similar significant decreases in twitch tension (P_t), maximum rate of twitch and tetanic tension development (Max.dP/dt) as well as increases in twitch time to peak tension (tpt) and $\frac{1}{2}$ relaxation time ($\frac{1}{2}$ rt) when compared with control (III) muscles. The twitch-tetanus ratio, specific muscle tension and fatigue characteristics of the reinnervated muscles did not differ from controls. Stimulated muscles were significantly larger and had a significantly higher mean maximum tetanic tension (P_o) than their unstimulated counterparts.

Group	I	II	III
Muscle wt (g)	2.30 ± .25[a]	1.89 ± .15[ab]	2.99 ± .34
Pt (N)	3.52 ± .26[a]	2.99 ± .01[a]	4.49 ± .99
Po (N)	25.7 ± 1.8	21.1 ± 1.5[ab]	28.1 ± 3.5
tpt (ms)	20.1 ± 1.0[a]	19.6 ± 0.5[a]	17.5 ± 1.5
1/2 rt (ms)	13.1 ± 0.5[a]	13.7 ± 1.4[a]	11.4 ± 0.8
dP/dt (N/ms) Pt	0.27 ± .04[a]	0.22 ± .04[a]	0.41 ± .08
dP/dt (N/ms) Po	0.49 ± .03[a]	0.42 ± .05[ab]	0.71 ± .07

Means ± s.d. [b] denotes sig. diff. ($p < 0.05$) I vs II; [a] denotes sig. diff. ($p < 0.05$) from III.

Thus, direct low frequency electrical stimulation performed from the day following denervation until after reinnervation has a beneficial effect on muscle weight and maximal tetanic tension. These changes occur with no evident alteration in the intrinsic speed or fatigue characteristics of the stimulated muscle.

190.15 DEMYELINATION OF MUSCLE AFFERENT AXONS FOLLOWING VENTRAL RHIZOTOMY IN THE CAT. J.N. Weakly and T.W. Bouldin*. Depts. of Physiology and Pathology, Univ. of North Carolina at Chapel Hill, Chapel Hill, N.C. 27514

Intact myelinated sensory axons arising from the medial gastrocnemius (MG) muscle were examined in teased-fiber preparations following unilateral interruption of ventral spinal roots (L6-S1) supplying the motor innervation to the muscle. Sensory axons appeared normal for up to 1½ mo., after which abnormalities of the myelin sheath became evident. In 12 of 14 cats observed 3-6 mo. after ventral rhizotomy, 18.0% (mean; range, 3.3-89.9%) of sensory axons in the distal 1 cm of the ipsilateral MG nerve (just proximal to the muscle entry zone) showed paranodal and/or segmental demyelination. Less than 1% of control axons in the contralateral MG or ipsilateral sural (cutaneous sensory) nerves showed such abnormalities. The demyelination showed a proximo-distal gradient: 10.9% (mean, range, 2.3-33.0% in 5 cats) of MG sensory axons 3-7 cm proximal to the muscle entry zone were demyelinated.

No myelin-sheath abnormalities were seen in ipsilateral MG or sural axons 3-6 mo. after section of L6-S1 dorsal roots, indicating that demyelination did not result from damage to the central processes of sensory axons.

No abnormalities were observed in intact MG motor axons following removal of L6-S1 dorsal root ganglia and consequent degeneration of sensory axons. Moreover, demyelination was rare (10/270 axons in 3 animals) in the few sural axons which remained after dorsal root ganglionectomy. These results and the delayed onset of the demyelination suggest that the abnormalities in sensory axons following interruption of MG motor axons are not related to the degeneration process per se.

The demyelination of MG sensory axons was observed in animals in which there was no physiological or morphological evidence of regenerating motor axons. When regenerating axons were present, the incidence of demyelinated axons was not increased over values in nerves without regenerating axons. Thus, it appears unlikely that the myelin abnormalities observed in intact axons were induced by signals from nearby regenerating axons.

In summary, severance and degeneration of motor axons in a mixed nerve and consequent muscle inactivation are associated with demyelination of intact sensory axons arising from the muscle. The results raise the possibility that normal axon-Schwann cell relationships depend on the frequency or pattern of sensory discharge or on impulse-independent signals arising from the paralyzed muscle.

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190.16 FUNCTIONAL AXON SPROUTING AFTER PARTIAL DENERVATION OF RAT HINDLIMB MUSCLES: EFFECT OF PREVIOUS EXERCISE TRAINING. P. F. Gardiner, J. L. Iadeluca*, and R. Michel*. Department of Physical Education, Université de Montréal, Montréal, Québec, H3C 3J7.

In several mammalian models, partial denervation of skeletal muscles is followed rapidly by sprouting of remaining intact axons which subsequently innervate muscle fibers denervated by the lesion (Pestronk et al., 1980; Brown et al., 1980). Since it has been previously suggested (Gerchman et al., 1975; Roy et al., 1978) that several characteristics of motoneurone metabolism and function may change as a result of chronic exercise training, a study was initiated to describe the effects of previous exercise training on functional axon sprouting in rat hindlimb muscles following partial denervation. Female Sprague-Dawley rats were assigned to control (C), partially denervated (D), or trained followed by partially denervated (T) groups. Animals in (T) were trained daily for 10 weeks on a rodent treadmill (26.8 m/min, 1 hour, 15% incline). In groups (T) and (D), partial denervation of triceps surae was accomplished by removing a 1 mm segment of L4 radicular nerve. In (T) this was performed the day following the final training session. Ten days following the lesion, soleus (SOL) and plantaris (PL) contractile responses to L5 radicular nerve stimulation were recorded in situ at 37°C. In (C) animals, L5 contributed means of 44% (SOL) and 25% (PL) of total muscle twitch tension. Both partially denervated muscles showed evidence of functional sprouting, in that L5-evoked tensions in gps D and T were displaced to the right of C. In PL of gp T, L5-evoked tensions were higher, with only this group possessing cases (17%) where tension exceeded 300 g/g. No comparable trend was evident in SOL. The data suggest that exercise training

L5-evoked twitch tension (g/g contral. muscle)[a]

	<100	101-200	201-300	301-400	401-500	>500
SOL C(10)	20	60	10	10	--	--
D(13)	--	8	8	68	8	8
T(11)	9	9	46	18	18	
PL C(10)	70	30	--	--	--	--
D(13)	23	46	31	--	--	--
T(12)	17	25	42	17	--	--

[a] Values represent % of total sample in each group

induces motoneurone changes conducive to enhanced axon sprouting after partial denervation. Differences between SOL and PL may represent differences in degree of overload, or differences in responses of nodal (PL) and terminal (SOL) sprouting mechanisms to overload.

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191.1 AFFERENT AND EFFERENT CONNECTIONS OF THE INFRAGRANULAR LAYERS OF RAT VISUAL CORTEX. Russell G. Carey and Teresa L. Neal*. Div. of Neurobiol., Barrow Neurol. Inst., Phoenix, AZ 85013.

We have begun an investigation of the laminar organization of the subcortical projections to cortex in the rat and recently reported differential projections to the supragranular and infragranular layers of parietal cortex (Rieck & Carey, 1982). The present report will present our initial results of a similar study in the rat visual cortices and focus on the afferent and efferent projections of the infragranular layers. Discrete electrophoretic injections of conjugated WGA/HRP were made into area 17 and into the medial and lateral parts of area 18 (18M and 18L). Control injections were made in the granular and supragranular layers in each cortical area.

After injections into each area distinct patterns of labeled cells and/or terminal grains were observed in many subcortical regions including the superior colliculus, lateral posterior nucleus (LP), posterior nucleus (Po), the dorsal lateral geniculate (LGN), lateral dorsal nucleus (LD), intralaminar nuclei, and the claustrum. In the superior colliculus only the projection from area 17 terminates in the superficial layers, while area 18L terminates in the intermediate layers and area 18M terminates in a multi-layered pattern in the intermediate and deep layers. Both area 17 and 18L receive a projection from the LGN; however, area 18M does not. Each of the cortical areas maintain reciprocal connections with at least two regions of LP and at least one region of Po and LD. Each of these regions form a column of cells/terminals that course through the thalamus in a rostral-caudal dimension. The projection of area 18M is particularly distinct and terminates heavily in two separate zones within LP that extend nearly throughout the entire rostral-caudal extent of this nucleus. Further, of the three cortical areas only area 18M has dense reciprocal connections with the claustrum. Area 18L appears to receive a moderate projection from the claustrum but does not appear to project to the claustrum.

There is, however, a remarkable similarity in the pattern of labeled cells found within the intralaminar nuclei of the rostral thalamus regardless of which cortical area is injected. These labeled cells form a continous slender band of cells that extend from the central nucleus to the lateral dorsal nucleus and lie in the medullary lamina that courses between the anterior ventral nucleus and the ventral anterior nucleus and surround the anterior medial nucleus. A second set of labeled intralaminar cells can be traced through the medullary lamina, lateral to the medial dorsal nucleus, to the superior central lateral nucleus lateral to the habenula.

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191.2 FUNCTIONAL CHARACTERISTICS OF THE VISUAL CORTICAL INPUT TO PARAFLOCCULAR PROJECTION ZONES OF THE BASILAR PONS OF THE RAT. C.E. Adams and D.J. Woodward. Departments of Cell Biology and Physiology, University of Texas Health Science Center, Dallas, TX 75235.

Previous investigations in this laboratory have described a visual cortical input to the paraflocculus via rostral lateral, ventrolateral, and dorsolateral basilar pontine regions. The aim of the present work was to examine functional characteristics of transmission in the visual cortico-basilar pontine component of this overall system. These studies are intended as preliminary to a developmental study of emergence of function in a system which has been shown to exhibit anatomical plasticity during ontogeny (Adams et.al., Neurosci. Abstr. 1981 #61.8).

Single units were recorded in the basilar pons of halothane anesthetized rats. The ipsilateral visual cortex (layer V) and contralateral paraflocculus were stimulated with concentric bipolar electrodes (David Kopf). Paraflocular stimulation provided for antidromic identification of pontine projection cells. Stimulation intensities ranged from 0.060-1.0 mA. Within the visual cortex, the lowest threshold response of pontine units was elicited by stimulation of the 17-18a border zone.

Response latencies of pontine projection cells fell into two groups. One (fast) group responded at latencies of 4-8 msec., while a second group (slow) was characterized by latencies of 8-12 msec. These two subgroups of pontine projection cells also differed in their ability to follow visual cortical stimulation at various frequencies. Cells within the "fast" group were found to follow stimulation frequencies of up to 20 Hz. Cells within the "slow" group exhibited a marked decrease in following capability at 15 Hz or above. Those pontine units which were responsive to visual cortical stimulation but unresponsive to antidromic stimulation exhibited similar dual latency groups and a slow-fast dichotomy was found as well for latencies of widespread pontine units activated by internal capsule stimulation.

These findings suggest that a dual projection system may exist within discrete cortico-pontine pathways of the rat, in this case, the visual cortico-ponto-paraflocular pathway. Similar data has been reported previously for the cat (Allen et.al., 1975b). A study of development of transmission in the visual cortico-ponto-paraflocular pathway is in progress.

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191.3 IDENTIFICATION OF VISUAL CORTICAL AREAS THAT PROJECT TO THE SUPERFICIAL OR DEEP LAYERS OF THE SUPERIOR COLLICULUS IN CATS. Richard L. Segal*, Stephen B. Edwards and Robert M. Beckstead, Dept. of Anatomy, Schl. of Med., Univ. of Virginia, Charlottesville VA 22908.

There are at least fourteen visual field representations in the parieto-occipital neocortex of the cat's brain. Many of these areas are known to send axonal projections to the superior colliculus (SC) and to influence the visuomotor function of SC. Mainly because the SC has been considered as an entity, studies to date have provided little data on even the crude segregation of these cortical afferents in the tectal laminae. We have successfully identified which of these cortical areas project to the superficial (visual) or the deep (sensorimotor) portions of SC by placing small deposits of either horseradish peroxidase (HRP, Sigma IX) or wheat germ agglutinin conjugated HRP (WGA-HRP) that are restricted to layers either above or below the stratum opticum. The ipsilateral retrograde labeling of cells (which are always pyramidal cells of layer V) in the various visual cortical areas is not uniform. Whereas areas 17, 18 and 19 contain the most HRP-positive cells after a superficial SC injection, the most numerous cell-labeling after a deep enzyme deposit occurs in the posterior lateral suprasylvian areas (medial, PMLS and lateral, PLLS). Another region that contains a substantial number of HRP-positive cells after a deep tectal enzyme deposit is the newly identified anterior ectosylvian visual area (AEV). Tectal projections from the other visual areas appear to be less preferential in their laminar distributions such that these areas (anterolateral, antermedial, dorsal and ventral lateral suprasylvian areas and area 21a) contain small numbers of labeled cells after any tectal HRP deposit regardless of location or apparent volume. Area 20 appears to totally lack a corticocollicular projection since the enzyme deposits consistently fail to label neurons in this area. The contralateral, cortical cell-labeling is sparser and less extensive than the ipsilateral and located most prominently in PMLS, PLLS, and AEV. That the input from the primary visual cortex (areas 17 and 18) is directed mainly if not exclusively to the superficial layers is consistent with earlier reports. Not previously appreciated, however, is the present observation that whereas several visual areas contribute a sparse input to the deeper collicular layers, this sensorimotor region of the tectum is probably most strongly influenced by the posterior lateral suprasylvian and, perhaps to a lesser degree, by the anterior ectosylvian visual area.

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191.4 SOME NOTES ON THE ULTRASTRUCTURE OF THE NUCLEUS OF THE OPTIC TRACT OF THE CAT WITH SPECIAL REFERENCE TO THE CORTICOPRETECTAL PROJECTION. B. Hutchins and J.T. Weber. Tulane University Medical School, New Orleans, La. 70112.

Both the normal morphology of the pretectal nucleus of the optic tract (NTO) and the visual cortical projections to the NTO have been examined at the ultrastructural level in the cat. Cortical terminals were identified with the aid of the anterograde transport of the enzyme tracer horseradish peroxidase (HRP). Large multiple injections of either wheat germ agglutinin-HRP (WGA-HRP) or Sigma Type VI HRP were placed within visual cortical areas 17 and 18 concurrently. Following a 48 hour survival period, the cats were perfused transcardially with 3% glutaraldehyde and 0.5% paraformaldehyde. 100 µm sections were cut on a vibratome with alternate sections being reacted with tetramethylbenzidine (TMB) for the identification of cortical terminals at the light microscopic level. The remaining sections were reacted with diaminobenzidine (DAB) intensified with cobalt chloride. The areas containing cortical terminals in the DAB reacted tissue were identified by direct comparison with the TMB sections. These DAB reacted regions were subsequently dissected and processed for routine electron microscopy. Since there is considerable confusion regarding the ultrastructural appearance of transported HRP, other areas, such as the lateral geniculate nucleus which is known to contain HRP labelled terminals following an injection of the visual cortex, were used for comparison.

Preliminary observations of the normal ultrastructure of the NTO reveal that numerous axonal presynaptic elements are in contact with several cell somas. Occasionally there are axo-axonal synapses, however, the greatest number of synapses are typically axo-dendritic. Of the axo-dendritic synapses, the postsynaptic elements are usually dendritic shafts. No synaptic specialization such as synaptic triads or glomeruli are observed.

Our experimental results provide confirmation at the ultrastructural level, of the controversial projection from the visual cortex to the NTO of the cat. The anterograde transported HRP for both WGA-HRP and the non-conjugated HRP appears consistently as small pleomorphic structures with multiple densities. The HRP labelled presynaptic elements within the NTO contain clear round or pleomorphic vesicles often associated with symmetric synaptic thickenings and pale mitochondria. Our initial observations indicate that the axon terminals range in size from small to large and are only in contact with dendritic processes. These latter dendritic processes also range in size from small to large.

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191.5 VISUAL CORTICAL INPUTS TO RETINAL VS NONRETINAL RECIPIENT ZONES OF THE PRETECTAL COMPLEX OF THE CAT. J.T. Weber and B. Hutchins. Department of Anatomy, Tulane University Medical School, New Orleans, La. 70112.

The visual cortical projections to the pretectal complex of the cat were investigated using retrograde tracing methods. Iontophoretic injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) were placed within the pretectum and the tissue processed according to the protocol for tetramethylbenzidine incubation (Mesulam, J. Histochem. Cytochem., '78). In all experiments, the retinae were removed and processed for WGA-HRP reaction product. Retinal recipient zones of the pretectal complex were determined on the basis of the presence of WGA-HRP labelled ganglion cells. The cortical maps of Tusa and colleagues (Br. Res., '78; '79; '80) were used to identify specific visual cortical areas.

Injections of WGA-HRP placed within the retinal recipient zones of the pretectum result in the labelling of neurons primarily within visual cortical areas 17, 19, 20A, 20B, PLLS, ALLS and PMLS. The labelled neurons are predominantly pyramidal and are located within layer V. Occasionally small ovoid labelled neurons, located in layer VI, are found within cortical areas 17, 19, 20B and PMLS.

When the injections of WGA-HRP are placed within the non-retinal recipient zones of the pretectum, labelled cells are found primarily within visual cortical areas 17, 18, 19, 20B, 21A, 21B, VLS, DLS, and ALLS. These latter labelled neurons are always located within layer V and are of the pyramidal cell type.

In both types of experiments (i.e., cases in which injections were placed within the retinal and nonretinal recipient zones of the pretectal complex), numerous labelled neurons are also seen within a region on the medial bank of the hemisphere which is ventral to area 17. Graybiel and Berson (M.I.T. Press, '81) have defined this latter region as the ventral splenial cortex.

Our results suggest that at least a part of the nonretinal input zones of the pretectal complex can now be considered visual by virtue of their cortical input. Further, depending upon the injection site, the difference in the areal and laminar distribution of cortical projecting neurons to the pretectum suggests that there are different functional areas within the complex.

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191.6 CORTICAL COOLING DEPRESSES VISUAL NEURONAL RESPONSES IN THE TECTORECIPIENT ZONE OF THE CAT'S LATERAL POSTERIOR NUCLEUS. Michael J. Hughes and Leo M. Chalupa. Department of Psychology, University of California, Davis CA 95616.

The medial zone of the cat's lateral posterior nucleus (LPm) receives a strong input from the superior colliculus. Recently, we (Chalupa et al., 1981) documented the visual receptive field properties of LPm neurons in cats maintained on a mixture of nitrous oxide and oxygen, supplemented with small doses of chloralose. In the present study the same type of preparation was employed to determine the degree to which the cortex contributes to the receptive field characteristics of LPm neurons. The LPm region was identified with acetylcholinesterase histochemistry (Graybiel and Berson, 1980), and the visual response of 59 cells within the tectorecipient zone were studied before, during and after cooling of the cortex. The portion of the cortex which was cooled included areas 17, 18, 19 and the LS visual areas. Our main finding is that during cortical cooling the visual responses of 88% of the units in LPm were markedly attenuated or totally abolished. In the cells which responded to stimulus movement as well as to flashed stimuli, the response to both stimulus conditions was equally depressed. The few cells which were unaffected by cortical cooling did not differ in terms of their receptive field properties or loci within LPm from the cells whose responses were depressed. In contrast, cortical cooling had a less pronounced and a more selective influence upon the visual responses of cells isolated in the main laminae of the lateral geniculate nucleus. These observations indicate that in the anesthetized cat the cortical inputs to the tectorecipient zone of the lateral posterior nucleus play a major role in the functional organization of this region. However, at present it is not known if this is due to the direct and/or indirect cortical projections to LPm.

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191.7 CORTICAL VISUAL INPUT TO THE MACAQUE PONS: THE TOTAL PROJECTION M. Glickstein, P. Inkpen* J.G. May III* and B. Mercier* (SPON: Charles Elbaum) MRC Unit on Neural Mechanisms of Behaviour 3 Malet Place, London WC1E 7JG U.K.

In previous experiments (Glickstein et.al. J. Comp. Neurol. 190: 209, 1980) we determined the cells of origin of monkey visual cortico-pontine projections by placing small injections of horseradish peroxidase (HRP) in the pontine nuclei and using di-amino benzidine for reacting cortical sections. In the present study we made much larger injections of HRP throughout the entire pontine nuclei and reacted cortical sections with the more sensitive tetra-methyl benzidine technique. We did these experiments to confirm and extend earlier findings about the origins of visual cortico-pontine projections.

The pontine nuclei were first located electro-physiologically after which a micro syringe was directed to the pons through the cerebellum of three monkeys in a horizontal stereotaxic plane. Large volumes (total of 2 to 4 μ litres) of HRP were placed in the pons by a series of multiple small injections. In two cases the injections were unilateral, in a third case, bilateral. HRP thus filled all of the pontine nuclei on one or both sides, and is likely to have been taken up by cortico-bulbar and pyramidal tract as well as cortico-pontine fibers. Confirming earlier work, there was a continuous group of labelled layer V pyramidal cells from the superior temporal to the intraparietal fissures on the dorsolateral aspect of the brain. Medially, this group was continuous at the level of the parieto-occipital notch with another large group of filled cells on the medial face of the hemispheres. This medial group extended from the splenium of the corpus callosum to the rostral bank of the parieto-occipital sulcus. Nearly all of the neurons which were found in these cortical areas, all of which are known to contain visual cells, were located in Brodmann's areas 19 or 7. Few cells were labelled in other cortical visual areas of the occipital or temporal lobe. There is a small projection from the rostral bank of the lunate sulcus. Confirming W. Fries' autoradiographic study (Neurosci. Abst. 7: 762 1981) there is also a small cortico-pontine projection from the striate cortex itself. Filled cells were found on the upper bank of the calcarine fissure with the greatest number in a region representing the extreme periphery of the lower visual fields.

Results suggest that cortico-pontine projections arise principally from those extra striate areas concerned with visual guidance of movement. Other prestriate areas and temporal lobe areas which are involved in visual form learning send far fewer fibers to the pons.

191.8 MORPHOLOGICAL FEATURES OF CORTICO-GENICULATE AXONS IN THE CAT. J. A. Robson. Dept. of Anat., SUNY Upstate Med. Center, Syracuse, NY 13210.

This study examines the morphological features of cortico-geniculate axons in normal adult cats. Horseradish peroxidase was injected into the optic radiations near the dorsal lateral geniculate nucleus in order to densely label both thalamo-cortical relay neurons (retrogradely) and cortico-geniculate axons (anterogradely). Light and electron microscopic techniques were then used to study the distribution, branching patterns and synaptic organization of the labeled axons within the nucleus.

Two types of axons can be seen in this material. Both types enter the nucleus dorsally and are oriented roughly along the "lines of projection." Both also cross laminar borders and can contribute terminal swellings to more than one lamina as well as to interlaminar zones. However the terminal arbors of these two types differ in their branching patterns, the distribution of their terminal swellings, and the synaptic organization of their swellings. The most frequently encountered type of axon is extremely fine and has sporadically distributed synaptic swellings. Some swellings arise as thickenings along otherwise smooth axons. Most, however, are at the ends of short collateral branches. The swellings are uniformly small with smooth surfaces and when examined electron microscopically they resemble the RSD terminals described by Guillery (Z. Zellforsch, 1969): that is, they are small (0.5-1.0 um); have round synaptic vesicles; and contain mitochondria with densely packed cristae. These terminals make asymmetric contacts usually onto small dendrites. In cases where labeled axons contact labeled relay neurons the synapes are onto peripheral dendrites. The second type of axon is far less common. These axons are characterized by their distinctive beaded appearance which is due to many closely spaced synaptic swellings. Swellings on short collateral branches are found occasionally but in general these axons have few branches. The synaptic swellings vary in size and shape. Some are small and resemble the RSD terminals described above. Others are larger (1.0-2.0 um). They have round synaptic vesicles and mitochondria with densely packed cristae and they form asymmetric contacts. However, they are frequently involved in synaptic arrangements involving more than one post-synaptic profile and, in addition to contacting peripheral dendrites, these terminals often contact proximal dendrites and somata. (Supported by NIH grant EY-03490 and the Alfred P. Sloan Foundation)

191.9 QUANTITATIVE ASPECTS OF THE CORTICO-GENICULATE PROJECTION IN THE CAT: A COMPARISON OF AREA 17 AND 18. H.C. Hughes. Department of Psychology, Dartmouth College, Hanover, N.H. 03755.

Cortico-geniculate projections are known to originate exclusively from layer VI of the visual cortex. In order to provide a more detailed description of these projections from areas 17 and 18, a quantitative analysis was performed. The data base for this analysis came from one cat which received a unilateral injection of horse-radish peroxidase (HRP) in the laminar LGN. The injection, which was delivered under hydrolic pressure via a recording micropipette, was clearly confined to the dorsal LGN and involved all the geniculate laminae. The topographic representation of the injection site extended from approximately 1° to 10° eccentricity near the horizontal meridian.

Sections (50μ) through area 17 and 18 were treated with O-dianisidine and counter-stained with thionine. Only sections containing the greatest density of retrograde labelling were selected for quantitative study. These densely labelled areas were reconstructed using camera lucida. The analysis included counts of labelled and unlabelled cells, and measurements of the soma diameters of labelled and unlabelled cells.

The results of this analysis lead to the following conclusions. 1) Cortico-geniculate cells tend to be concentrated in a deeper sub-lamina of layer VI (in layer VIb, cf. O'Leary, JCN 75, 1941). 2) In agreement with other reports, virtually all cortico-geniculate cells could easily be classified as pyramidal cells. 3) Area 17 contributes more than twice the number of cortico-geniculate cells than area 18 (average of 58 cells/200μ wide column versus 20 cells/200μ in area 18.) 4) Despite these numerical differences, areas 17 and 18 contain equivalent percentages of cortico-geniculate cells in layer VI. This is due to the fact that layer VI is wider and more densely populated in area 17. 5) The distribution of soma diameters of geniculo-cortical cells was equivalent in both areas (mean dia = 13.57μ for area 17 and 13.43μ for area 18). 6) Cortico-geniculate cells tend to be larger than other cells in layer VI (ave. dia. of unlabelled cells = 9.3μ).

These results lead to the expectation that area 17 exerts a more powerful influence over the LGN than area 18, but that it does so as a simple consequence of the greater number of cells in layer VI of the striate cortex, rather than by differences in the apportionment of layer VI cells within these areas.

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191.10 EFFECTS OF CRYOBLOCKADE OF VISUAL CORTEX ON RETINAL GANGLION CELL OUTPUT. S. Molotchnikoff, F. Tremblay* and F. Lepore. Dept of Biological Sciences, Université de Montréal, Montréal, Québec, Canada.

Does the visual cortex affect the neuronal firing of retinal ganglion cells? The question has been raised, but as yet it has not been answered. To test this hypothesis the visual cortex was inactivated by a reversible cold block in urethane anesthetized and paralyzed rats (N = 30). Action potentials in response to a short flash (1 H_z) were recorded from ganglion cell axons at the level of the optic tract prior to, during and following cortical blockade. Antidromic spikes evoked by applying an electrical pulse to the lateral geniculate nucleus confirmed the axonal origin of the recordings. In initial experiments (N = 10) we recorded field potentials to examine how the waveform was modified. Flash presentation elicited two positive peaks with implicit times of 50 and 80 msec respectively. The magnitude of the second wave was generally 50% that of the first. As the visual cortex was cooled, a significant increase in latencies and amplitude of the second peak was observed. The cortical blockade therefore appears to influence exclusively the late response.

In a second series of experiments single unit recordings were carried out. Results indicated that the visual cortex was strongly involved in modulating the discharge pattern of retinal ganglion cells. In 30% of the cells, there was an increase in bursting pattern and a concurrent occurrence of additional short and long latency discharges. Data were also analyzed in terms of inter-spike interval histograms (IH). This analysis showed that the distribution of retinal efferent impulses was under the control of a cortico-retinal feed-back. Since the B-waves of the ERG were not modified (double pulse technique) it was assumed that the retinopetal fibers act at the level of the inner plexiform layer.

The data support the notion that in mammals the visual cortex and the retina have reciprocal relationships.

Supp. CRSNG and FCAC.

191.11 HETEROTOPIC CALLOSAL PROJECTIONS AND THE DEVELOPMENT OF CALLOSAL AFFERENTS IN RAT VISUAL CORTEX, Michael Miller and Brent A. Vogt. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

Complete section of the corpus callosum in hooded rats causes axons to degenerate in contralateral visual cortex. Only the lateral one third of area 17 receives callosal afferents, whereas degeneration in secondary visual areas is limited to patches or strips of cortex. After a large injection of horseradish peroxidase involving much of area 17, retrogradely filled neurons are identified throughout contralateral area 17 and areas 18a, 18b, and 29d. Thus, primary visual cortex is connected to the contralateral hemisphere by homotopic (lateral area 17 to lateral area 17) and heterotopic (e.g. medial area 17 to lateral area 17) projections.

Homotopic callosal projections from lateral area 17 arise from neurons distributed evenly in layers II/III, IV, and V (24-34% in layer V) and terminate in a bilaminar pattern; layers I-III and layer V. In contrast, callosal connections which are heterotopic by cortical region are also heterotopic by layer. Heterotopic connections arise primarily from cells located in layer V (60-80%). and terminate in a bilaminar or unilaminar pattern. An example of the unilaminar termination pattern is the projection of layer V neurons in area 18b to supragranular layers in lateral area 17. Secondary visual cortex forms a network of heterotopic callosal connections, however, unlike area 17 only areas which receive callosal afferents seem to contain callosal projection neurons.

During development, before cortical areas can be distinguished on cytoarchitectonic criteria (first postnatal week), all of the posterior neocortex receives projections via the corpus callosum. These fibers cross the callosum and wait in the intermediate zone before entering the newly segregated cortical laminae on postnatal day 3. The paring down of callosal axons into the mature organization seems to occur first in area 17 (by day 9) and then in secondary visual cortex (day 12).

Heterotopic connections may be involved in forming binocular visual receptive fields in the rat, and possibly in driving units with large, non-oriented receptive fields described in layer V of rodent primary visual cortex. These callosal connections appear to be established before the eyes of rat pups open at the end of the second postnatal week. Supported by grants EY 07054, NS 07016, and NS 18745.

191.12 EFFECT OF HEAD TILT ON THE VISUAL EVOKED RESPONSE TO A MOVING LINE, S. Reinis, R.H. Lahue*, D.S. Weiss*, J.P. Landolt and K.E. Money*. Dept. of Psychol., Univ. of Waterloo, Waterloo, Ont. and DCIEM, Downsview, Ont. Canada.

In previous work, we reported that the basic characteristics of most visual cortical complex cells changed when the head of the experimental animal was tilted to the right and to the left (Soc. Neurosci. Abstr. 6: 578, 1980; 7: 174-175, 1981). In this paper, we present data showing that the cortical visual evoked responses to a moving line change as well. Cats with surface recording electrodes implanted over the left and right visual cortices were immobilized with Pavulon® and a line was projected onto a screen placed 81 cm in front of their eyes. The line was moved at 30°/s, at angles of 0°, 45°, 90°, 135°, 180°, 225°, 270°, 315° and 360°, relative to the horizontal. The response to individual angles differed in the number and amplitude of the evoked waves at the onset of the stimulus, in the degree of synchronization during the movement of the stimulus across the screen, and in the number and amplitude of the waves following termination of the stimulus. Tilting the head by 45° to the right or to the left altered the character of the response in relation to the subjective horizontal. Also, an intravenous injection of deuterium oxide (4 ml/kg) altered the response to the moving line. These data further indicate that stimuli from the vestibular system and/or from the neck proprioceptors contribute to the analysis of the visual stimulus by the visual cortex. Supported by DCIEM Research Contract 97711-0-5951/8SE80-00185.

191.13 VISUAL CORTICAL CELL CHANGES FOLLOWING THE ADMINISTRATION OF NITROUS OXIDE, R.H. Lahue*, S. Reinis, D.S. Weiss*, J.P. Landolt and K.E. Money*. Dept. of Psych., Univ. of Waterloo, Waterloo, Ont. and DCIEM, Downsview, Ont. Canada.

Single cell activity in the central nervous system of cats has often been recorded in animals anaesthetized with nitrous oxide (N_2O). In these studies, it was tacitly assumed that the anaesthetic did not change the normal function of the cells that were studied. In order to assess the suitability of this anaesthetic for this type of research, we compared the characteristics of individual complex cells in the visual cortex before and during the administration of 25% N_2O - 75% oxygen mixture. (Animals were previously prepared under anaesthesia for microelectrode recording stereotaxically according to procedures reported earlier in Ann. N.Y. Acad. Sci. 374: 262-273, 1981.) We found that, in more than 80% of the cells, after the system was saturated with nitrous oxide, the optimal length of the moving line stimulus eliciting the response in the complex cell changes together with the area of its receptive field. The spontaneous intertrial activity of the cells changed as well. The directional preference and localization of the receptive fields did not change. In about 10% of the cells, the receptive field disappeared completely and re-appeared within 30 minutes after the N_2O was discontinued. These data indicate that N_2O modifies the characteristics of visual cortical cells in several ways, and, therefore, as an anaesthetic, it is not suitable for electrophysiological studies of this type. Supported by DCIEM Research Contract 97711-0-5951/8SE80-00185.

191.14 MONOCULAR LOSS, BINOCULAR MAINTENANCE OF PERCEPTION IN GANZFELD. S. J. Bolanowski, Jr. and Robert W. Doty. Center for Brain Research, University of Rochester Medical Center, Rochester, New York 14642.

We have found that when human observers (n=20) monocularly view a uniformly lit, contourless visual field, a Ganzfeld, a dramatic loss of perception or "blankout" begins after about 12 sec. During a 1-min period of steady viewing the "blankout" may subside briefly, sometimes in association with blinking or eye movements, but is present for a substantial proportion of the viewing time. Most surprisingly, when the Ganzfeld is viewed binocularly, this loss of visual perception does not occur, even when viewed for over 3 min.! The "blankout" process was reported by every observer to resemble a perceptual curtain, originating nasally and being drawn naso-temporally across the visual field. All subjects also described the persistence of perception in an area of the far temporal visual field into which "blankout" did not seem to project. This area corresponds to the "temporal crescent", the region within the visual field which is seen with only one eye. In addition, preliminary experiments suggest that the consensual pupillary reflex follows the "blankout" process, slight dilatation occurring during the periods of perceptual loss.

We have presented our dark adapted subjects colored and achromatic Ganzfelden (matched for equal luminance, 0 N.D. = 16.2± 1.2 cd/m^2 and variable in 1.0 log unit intensity steps) pseudorandomly to the right, left and both eyes. Each observer was asked to signal the onset of "blankout" and its duration by pressing a response key. On average, only negligible differences in response latency occurred between eyes and only small differences (<3 sec) were found among stimulus hues. The intensity of a monocularly presented Ganzfeld did not significantly affect latency for "blankout", suggesting that the mechanism mediating "blankout" onset operates across photopic, mesopic and scotopic levels, although the percentage of time in "blankout" does decrease with increasing intensity. Additional experiments in which binocular Ganzfelden were presented, constant intensity to one eye and variable intensity to the other, show that "blankout" does not occur if disparities in light intensity to the two eyes are within 2 log units.

These results indicate that central as opposed to retinal processes play a major role in mediating "blankout" and that the mechanism responsible for this phenomenon operates with a processing time of about 12 sec regardless of intensity or hue. Persistence of perception in the "temporal crescent" and under binocular viewing conditions are consistent with our hypothesis that the "blankout" arises from processes similar to those underlying binocular rivalry. Single unit activity has been observed in striate cortex of unanesthetized macaques which is concordant with these psychophysical effects (Kayama et al. J. Neurophysiol. 42: 1495, 1979).

192.1 MORPHOLOGY OF STELLATE CELLS IN LAYER IV OF CAT AREA 17. M.D.Kay*, J.F.Dashe*, W.H.Mullikin*, and T.L.Davis, Dept. Anat., School of Medicine, Univ. of Penn., Phila., Pa. 19104.

Reconstruction of synaptic patterns on somas and proximal dendrites of neurons in layer IVab suggested a diversity of cell types (Davis and Sterling, J.Comp.Neur.: 188,1979) . These results were supported by the finding of four classes of Gaba-accumulating neuron in layer IVab (Hamos et al, Soc. Neurosci. Abstr.:7,1981). It is not known, however, whether there is a corresponding diversity in the three-dimensional morphology of these neurons. We have begun to identify distinct morphological types of neurons in layer IV of the adult cat by obtaining Golgi-like filling of cells via intracellular or extracellular depositions of horseradish peroxidase. Filled cells were drawn using a camera-lucida and a 100X objective and the following morphological features noted: soma shape and size, number of primary dendritic branches, pattern and extent of dendritic arborization, and dendritic spine density.

So far, we have recognized eight morphological types of stellate cell in layer IV. Two types were aspinous, one having a large soma (21um) and smooth dendrites, the other having a medium soma (12 um) and varicose dendrites. Six types were spinous. Two of these had a small soma (9 um) one being sparsely branched and the other bushy. Three types had a medium, oval soma (11-12 um) but differed in the richness of their dendritic arborizations and spine densities. The eighth type had a fusiform soma (22 X 7 um) oriented tangentially to the pia and spiny dendrites extending longitudinally from the soma.

Since there is evidence that a given cell type in layer IV may represent but a small fraction of the total population (Solnick et al, Soc. Neurosci. Abstr.:7,1981) we anticipate finding additional morphological types. An important step will be to determine whether a cell type characterized by a distinctive morphology also has a distinctive pattern of synaptic input.

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192.2 THE NUMBER OF NEURONS IN THE DIFFERENT LAMINAE OF THE BINOCULAR AND MONOCULAR REGIONS OF AREA 17 IN THE CAT. C. Beaulieu*, M. Colonnier and J. Thibodeau*. Department of Anatomy, Laval University, Quebec, Qué. G1K 7P4.

The number of neurons in individual laminae of area 17 was determined for both binocular and monocular regions in the left hemisphere of six cats. For each region, portions unaffected by curvature were chosen, i.e., portions where the pial surface was relatively straight and in which the columns of cells and radial bundles of axons were strictly parallel to each other. The numerical density was determined for each lamina by using the method of size-frequency distribution, applied to neuronal nuclei (Weibel '69, Int. Rev. Cytol: 26, 266). Measurements of the area and of the maximum diameter of the nuclei were made with a graphic tablet on photographs (X1200) of 1 um epon sections stained with methylene blue. The number of neurons under 1 mm^2 of cortical surface and standard deviation are given in the following table, for both regions. The figures have been corrected for tissue shrinkage.

	BINO	MONO	p<
I	1242 ± 602	1299 ± 227	-
II	7634 ± 1192	6762 ± 971	.05
IIIA	8780 ± 1595	6833 ± 1758	.05
IIIB	10111 ± 1895	8780 ± 2602	-
IVA	12685 ± 2505	10104 ± 1650	.05
IVB	13796 ± 2487	12477 ± 1576	-
V	5915 ± 219	4312 ± 731	.001
VIA	15175 ± 1169	10513 ± 974	.001
VIB	2178 ± 678	1508 ± 586	.05
TOTAL	77516 ± 6160	62588 ± 5038	.005

p was calculated with a Mann-Whitney U test.

Note that the number of neurons under 1 mm^2 of cortical surface is significantly less in the monocular region for the total cortical thickness and that this is due to significant differences in layers II, IIIA, IVA and especially to changes in layers V and VIA. This suggests that in the binocular region there are possibly additional interneurons specifically related to binocular interaction, or that there is a greater number of neurons projecting to other cortical and to subcortical areas. There is little data in the literature to support one or the other alternative.

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192.3 STIMULUS SPECIFICITY OF BINOCULAR NEURONS IN THE CAT'S STRIATE CORTEX: A QUANTITATIVE COMPARISON OF RIGHT AND LEFT EYES B.C. Skottun* and R.D. Freeman, School of Optometry, University of California, Berkeley, CA 94720

A large majority (about 80%) of neurons in the cat's visual cortex can be driven by either eye. However, the relative strength of the input from the two eyes (ocular dominance) varies from cell to cell. We have asked whether the stimulus specificity for each eye is matched over this range of ocular dominance or varies in some systematic manner with relative interocular response strength.

Cats were prepared using standard procedures for extracellular recordings in the visual cortex (Area 17). After initial receptive field mapping with manually controlled stimuli, single cells were studied quantitatively using sinusoidal gratings presented on a specially designed TV monitor. Cells were classified as simple or complex according to the modulation of the response. The degree of modulation for each of the two eyes was generally highly correlated. On the other hand, for 9 out of 92 cells, modulation was different enough to suggest a "simple" category for one eye and "complex" for the other. Orientation preference was relatively similar in the two eyes, except for a small offset that we attribute to the bilateral interocular intortion of paralysed cats. Orientation selectivity (1/2 width at 1/2 height) was also highly similar (correlation r=0.73), although there was a slight tendency for tuning in the dominant eye to be broader. For spatial frequency, the two eyes were also well matched: optimal spatial frequencies and tuning bandwidth correlated with coefficients of r=0.92 and r=0.76 respectively. Finally, contrast-response functions varied from those that appeared linear (response vs log contrast) to those that exhibited saturation at low contrast levels. In most cases the shapes of the contrast-response functions were similar for the two eyes and the main difference was the slopes of the curves.

We conclude that the stimulus specificity for binocular striate cells is generally well matched for the left and right eyes and that this accordance is typically not dependent on ocular dominance. The results show these cells to be well suited for functions such as stereopsis.

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192.4 THE COMPLETE PATTERN OF OCULAR DOMINANCE STRIPES IN MACAQUE STRIATE CORTEX. M. Connolly*, S. Le Vay, and D.C. Van Essen. Biology Div., Caltech, Pasadena, CA and Neurobiology Dept., Harvard Medical School, Boston, MA.

Previous studies of the configuration of ocular dominance columns in macaque striate cortex have demonstrated an array of alternating left-eye/right-eye stripes over the operculum and in the roof of the calcarine fissure. We have examined the complete pattern of stripes in a single hemisphere, using autoradiography after transneuronal transport of ^{3}H-proline from the left eye. Recordings were made from several regions of cortex prior to perfusion in order to establish the approximate layout of the visual field representation in the cortex. Two-dimensional maps of the left striate cortex were prepared independently by two techniques: 1) the manual reconstruction technique of Van Essen and Maunsell (JCN 191: 255, 1980), and 2) a computerized method, involving the generation of a complete three-dimensional model of the pattern of columns, photography of this model at a variety of angles (to provide face-on views of all parts of striate cortex), and the assembly of a two-dimensional montage from these photographs.

The two reconstructed versions were similar in overall size and shape of striate cortex and in the layout of ocular dominance stripes, although there were numerous differences in detail. As expected from previous studies, the stripes intersected the striate boundary approximately at right angles. In the region of peripheral representation they tended to run orthogonal to the horizontal meridian representation, but they were not strictly parallel to iso-eccentricity contours. There were two unexpected findings: 1) The periodicity of the stripes was not constant. The width of a left- plus right-eye pair ranged from less than 0.5 mm to more than 2.0 mm, a more than four-fold range. The broadest spacings occured along the vertical meridian and the narrowest in the far periphery. 2) Beyond about 20 degrees eccentricity the columns for the contralateral eye were often two to three times wider than those for the ipsilateral eye, resembling the pattern found with monocular deprivation. This contralateral bias in the periphery was confirmed in the opposite hemsphere and in the brains of two other monkeys. Lastly, the representation of the optic disc was about twice as long as it was wide, consistent with the possibility of a significant anisotropy in the visual representation within layer IVc of striate cortex.

Supported by NIH grants EY R01-1960 and EY 02091.

192.5 CHOLECYSTOKININ-LIKE IMMUNOREACTIVE NEURONS IN RAT NEOCORTEX, Alan Peters and Michael Miller. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA., 02118

The distribution and form of cholecystokinin (CCK) immunoreactive neurons within the posterior pole of the rat cerebral hemisphere were studied using the immunoperoxidase technique. The antiserum obtained from Immuno Nuclear Corp. was directed against the carboxy terminal octapeptide of CCK.

There are CCK-positive neurons throughout the cortex, but they are most common in the supragranular layers. Comparing the shapes of these CCK-positive neurons with the forms of neurons revealed by Golgi impregnation, it is concluded that CCK-positive neurons are of three types. There are layer II/III bipolar cells which have very narrow and vertically elongated dendritic trees, layer I neurons which are both horizontal and multipolar, and other non-pyramidal cells. These other non-pyramidal cells can have either multipolar or bitufted dendritic trees, and although they are most common in layer II/III some are present in infragranular layers. The interpretation that all of the CCK-positive neurons are non-pyramidal is supported by electron microscopic study of some of these neurons, for all of those examined have both symmetric and asymmetric synapses on their perikarya, a characteristic feature of non-pyramidal neurons. It is also apparent in thin sections that the horseradish peroxidase reaction product forms a granular deposit throughout the cytoplasm and nucleoplasm, and shows no predilection for any particular type of organelle. By light microscopy three types of CCK-positive axons are apparent. These are vertically oriented axons believed to arise from the bipolar neurons, a plexus in layer II/III considered to arise from the multipolar and bitufted cells, and a plexus of coarse axons in layers V and VI derived from an unknown course.

Previous Golgi-electron microscopic studies have shown that axons of bipolar cells form asymmetric synapses, suggesting that they are excitatory neurons. In contrast, bitufted and multipolar neurons similar to the ones visualized by the CCK antibody have been shown to have axons forming symmetric synapses and to contain glutamic acid decarboxylase. Thus, although iontophoretically applied CCK excites cortical neurons, it appears to be present in some neurons which are excitatory and others which are inhibitory in function. (Supported by NIH grants NS 07016 and EY 07054).

192.6 TEMPORAL SUMMATION IN THE CAT VISUAL CORTEX. J. Duysens, G.A. Orban, J. Cremieux* and H. Maes*. Lab. Neuro- en Psychofysiologie, Katholieke Universiteit te Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

154 cells from the LGN and from areas 17 and 18 of paralyzed and N_2O anesthetized cats were examined in a search for the population of visual cortical cells responsible for the temporal low-pass filtering taking place at the cortex (Brenner et al., Soc. Neurosci. Abstr., 7:175, 1981). A stationary light bar was flashed over the most sensitive region of the receptive field for variable periods of time in a randomized order. The units were classified according to their qualitatively and quantitatively measured responses to moving or stationary presented stimuli (velocity-response curve and response plane).

It was found that the firing rate of the ON-responses of LGN cells (n=14) did not depend on the stimulus duration. In contrast, 30% of all cortical cells were seen either to increase their ON firing rate by more than 100% when progressing from the shortest (12.5 msec) to the longest (3200 msec) ON stimulation duration, or more frequently, to have a threshold ON duration, i.e. requiring a minimum ON duration which is longer than the shortest ON duration used. These cells, which apparently perform a temporal integration of LGN input, were more numerous in area 17 than in area 18, had mostly non-overlapping subregions (S or HS cells) and generally preferred low velocities. A combined velocity profile of all these cells showed a maximum response at the lowest velocity tested (0.5 deg.sec^{-1}) and a decrease to 50% of this maximum at 13 deg.sec^{-1}.

Cells that showed no sign of modulation of the ON response in function of changes in ON duration could be classified into two groups. In one group the ON response was either very weak or completely absent at all ON durations (34%). Some of these units, especially the S or HS cells, were found to have a threshold OFF duration when tested with variable OFF durations. The other group (36%) exhibited clear ON responses, independent of ON stimulus duration (as did units in the LGN). Units in this group belonged mostly to the C family (complex-like) and their average velocity profile showed very little velocity selectivity. It is concluded that for areas 17 and 18, the slow cells of the S family (simple-like cell) can be identified as the cell population specialized at temporally integrating visual signals coming from the LGN (Orban et al., this volume).

192.7 THE CORTICO-CLAUSTRAL LOOP CONTRIBUTES TO END-INHIBITION OF NEURONS IN AREA 17 OF THE CAT. Helen Sherk* and Simon LeVay (Spon: T. Sejnowski). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

We have recently described the response properties of neurons in the cat's visual claustrum, which is reciprocally connected with area 17 and other visual cortical areas (Sherk and LeVay, J. Neurosci. 1:993). They are orientation-selective, binocular and non-directional. A striking feature is their preference for very long bars or edges.

To determine the role of the return projection from the claustrum to visual cortex, we have recorded from neurons in area 17 after unilateral ablation of the visual claustrum with kainic acid. 462 cells were studied in area 17 in the deafferented hemispheres of six cats, and 636 cells in the intact hemispheres of these cats and in two other controls. All response properties were assessed qualitatively.

Properties studied included receptive field position and size, cell type (simple, standard complex, special complex, etc.), preferred orientation, sharpness of orientation tuning, direction selectivity, length summation, end-inhibition, preferred bar thickness, edge responses, ocular dominance, responsiveness and spontaneous activity. Tracks and marking lesions were reconstructed and each cell was assigned to a layer (1-3,4,5 or 6).

Only end-inhibition was affected by the claustral lesions. This was reduced on the side of the lesion in every cat. Of 442 cells in layers 1-3 and 4 of the control hemispheres, 42.5% showed moderate, strong or total end-inhibition, whereas only 19.3% of cells in the experimental hemispheres did so. Total end-inhibition was particularly rare on the side of the lesion: it was seen in only 13 cells (4.4%), compared with 78 cells (17.6%) on the control side.

The results suggest that the claustral loop makes a major contribution to the end-inhibition seen in visual cortex. Hubel and Wiesel (1965) originally suggested that cortical end-inhibition might be generated by inhibitory neurons that responded selectively to very long stimuli, and claustral cells fit this description. Since end-inhibition was not completely lost after claustral lesions, it is probable that intracortical mechanisms also play a role. (Supported by NIH EY-R01-1960).

192.8 STRIATE CORTEX: CONTRAST ADAPTATION INDUCTION AND RECOVERY. D.G. Albrecht, D.B. Hamilton*, and S.B. Farrar*. Dept. of Psychology, University of Texas, Austin, Texas 78712.

Prolonged viewing of a high contrast grating pattern produces a variety of perceptual effects; among others, the grating seems to gradually fade although not totally dissappear and the detection threshold increases. Converging psychophysical and physiological evidence suggests that striate cells play a fundamental role in these effects, presumably reflecting the underlying contrast gain control in the cortex.

We have been investigating the contrast adaptation properties of striate cells using stimuli similar to the psychophysical experiments to facilitate a comparative analysis. After first measuring the contrast response function (along with the receptive field plot, and spatial and temporal frequency tuning), we performed the following adaptation experiment. Each cell was stimulated by drifting its optimal spatial-temporal frequency grating pattern across the receptive field. The temporal sequence of events consisted of four intervals. (1) During the first (Pre-Adapt) 30 sec interval, the contrast was set to produce 5-10% of the cell's maximum response, to index pre-adapt sensitivity. (2) The next 120 sec consisted of a no-pattern rest interval. (3) After the rest, the contrast was set to produce the maximum response and was left on (Adapt) for 30 sec. (4) The adaptation was immediately followed (Post-Adapt) by a low contrast grating, presented for 30 sec at the pre-adapt contrast to index post-adapt sensitivity. This entire sequence of events was repeated from 4 to 8 times (with a 5 min wait between each repetition) to increase the reliability of the estimate.

Examination of the pattern of results (across cells) during the high contrast adaptation interval reveals several qualitatively different types of effects. Some cells produce their maximum response as soon as the high contrast grating is turned on whereas others actually take some period of time (1 to 20 sec) to reach their maximum. Once the maximum response of the cell is achieved, the response rate gradually declines, although there is a great deal of variation from cell to cell in the rate of induction: some cells reduce their response by 90% (to an asymptotic plateau) during the first 10 sec of adapt while others reduce their response by only 10% over the full 30 sec of adapt. (It may prove useful to characterize cells along a sustained transient dimension.) After the prolonged adaptation, contrast sensitivity was lower during the post-adapt interval, although once again comparing the rate of recovery across cells reveals a great deal of variation: some cells reach a recovery plateau in five sec while others still show strong adaptation after 30 sec.

192.9 TWO CONTRAST MECHANISMS REVEALED BY PATTERN VISUAL EVOKED RESPONSE IN THE ALERT MONKEY. M.Mackeben and K.Nakayama
Smith-Kettlewell Inst.of Visual Sciences, San Francisco, CA 94115

Steady state visual evoked potential (SSVEP) recordings were made from two macaque monkeys while they fixated and attended to a fixation light. Electrodes were implanted in the bone over pre- and post-lunate visual cortical areas (V4 and V1). The contrast of a sinusoidally counter-phase modulated gratings was varied between 1 and 82%. The responses were tested at a wide range of spatial and temporal frequencies. A contrast function run consisted of 78 trials (3 sec duration, 6 repetitions for each of 13 contrast values).

More than half of the 143 runs recorded in both monkeys yielded log contrast (C) vs. amplitude functions with two distinct linear limbs. While there was some overlap in the slope ranges (.3 to 1.5 μV/dB versus .6 to 4.0 μV/dB), for any one function the slope of the high C limb was always steeper than that of the low C limb. The appearance of the high C response was always accompanied by a change of slope in the phase characteristic, which indicates two different underlying mechanisms. The two limbs of the C function could be direct or indirect reflections of input from the parvo- and magnocellular layers of the LGN, which have been shown to have different contrast sensitivities (Kaplan and Shapley, J.Physiol., in press).

Extrapolating the low C limb to zero response amplitude gave us the theoretical thresholds, which were typically below 5% and above 10% for the low and high C limbs, respectively. Like in the human (Campbell and Maffei, 1970), calculating the contrast sensitivity from these values resulted in a good match with the psychophysically measured contrast sensitivity function of macaque monkeys (DeValois et al., 1974). Extrapolating the high C limb, however, did not produce such a match. In addition, we found that the extrapolated thresholds could vary with the temporal frequency of stimulus modulation. The narrow spatial frequency tuning reported earlier (Nakayama and Mackeben, Brain Res. 193, 1980) was found to be present only at high stimulus contrasts (Nakayama and Mackeben, Vis.Res., in press).

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192.10 RADIAL OR PINWHEEL MOTION EVOKES A STRIPED PATTERN OF ACTIVITY ON THE VISUAL CORTEX SURFACE. M. Cynader and C. Shaw. Dept.'s Psychol. & Physiol., Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

Staring at the center of a rotating logarithmic spiral produces a powerful sensation of motion toward or away from the observer. The geometric basis of this motion in depth has been considered by Gibson, Koenderink, Regan & Beverley, and others. Here we use the C^{14}-deoxyglucose (2DG) method to explore the pattern of activity evoked in the visual cortex by stimulation with radial motion (both inward and outward) and compare it with that evoked by having animals fixate the center of a pinwheel (rotating clockwise and counterclockwise). This latter stimulus has no depth component associated with its motion.

Paralyzed 3-4 month old kittens viewed the spiral or pinwheel patterns binocularly for 30-40 minutes after I.V. administration of 100 μ Ci/kg of 2DG. After perfusion, visual cortex on one side was removed and carefully flattened between glass slides in order to provide a large surface over which 2DG uptake could be measured. The cortex was sectioned parallel to the surface (tangential sections). The sections were used to expose X-ray film. The other hemisphere was cut sagittally.

Both tangential and sagittal sections revealed that viewing a moving spiral pattern evoked a regular pattern of alternating stripes on the visual cortex surface. The stripes have a period of about 1200 μ and width of 600 μ. The stripes run medial to lateral, approximately perpendicular to the border between areas 17 and 18. The pattern of cortical 2DG uptake evoked by viewing a rotating pinwheel is similar to that evoked by radial motion. Again one observes a regular pattern of stripes running medio-lateral across large expanses of the cortex.

We compared the organization of these patterns of evoked glucose consumption with those resulting from stimulation of (1) one eye alone with stimuli of varied orientations, directions, and velocities, and (2) both eyes with a stimulus pattern of one orientation only, moving in both directions with varied velocity. Both monocular viewing and viewing of contours of one orientation evoked substantial inhomogeneities in the pattern of 2DG uptake on the cortical surface. In the case of monocular exposure, patches formed on the cortical surface without substantial tendency for elongated bands. With stimulation at one orientation, the pattern of activity was more regular, with zones of locally parallel bands, but the overall clarity and consistency of the pattern evoked by radial or pinwheel motion was absent.

The remarkably clear and simple patterns of cortical activity evoked by the spiral or pinwheel patterns suggest that the information they convey is an important feature of cortical processing mechanisms.

192.11 PROCESSING CAPABILITY OF THE PRIMARY VISUAL CORTEX AND POSSIBLE PHYSIOLOGICAL BASIS FOR AN APPARENT MOTION ILLUSION. Gordon L. Shaw, Physics Dept. U. of Calif., Irvine, CA 92717, Patricia C. Rinaldi, Div. of Neurosurgery, U. of Calif., Irvine and John C. Pearson*, Physics Dept., U. of Calif., Irvine.

The primary visual cortex is known to process stimuli in a given part of the visual field such that divisions of the cortical column into orientation and ocular dominance minicolumns are present. Applying a model incorporating both the Hebb learning hypothesis and a Mountcastle-like organizational structure to the visual cortex, we predicted that the processing capabilities of the column involve dynamic interactions among minicolumns and are much greater than presently documented. In particular, processing of rotational stimuli in area 17 was suggested by the model. Motivated by this, a psychophysics experiment was performed which demonstrated a spatial-temporal filling in process in apparent motion. We have now presented this "human illusion" to cats. Stimuli were presented to cats maintained under N_2O and flaxidel. Extracellular microelectrodes were placed in area 17 with the aid of stereotaxic coordinates and isolated single units were observed. The center of the visual field and optimum bar orientation were determined for the monitored neuron, and then repeated time sequences corresponding to "clockwise" and to "counterclockwise rotations" of the stimuli were presented, both with and without the optimum stimulus orientation. Some representative, preliminary data are presented. Although the "filling in" process has not as yet been clearly observed, a number of striking features have been found. For example: a) We observed dramatic sharpening as well as greatly increased peak firing response when the optimum stimulus is left out of the sequence (for one cell, the width of the post-stimulus histogram at the time of the second bar presentation decreased from 30 ms to less than 4 ms). b) Some cells show two activity peaks or even four peaks of large size when only one bar orientation is shown. However, when a time sequence of other orientations is shown, then some of these peaks are suppressed. c) We noted a striking change in firing activity dependent soley on the change of the sense of presentation from "clockwise" to "counterclockwise". We have found no simple explanation (such as "lateral inhibition") which will explain these phenomena.

192.12 RESPONSE OF CAT'S GENICULATE AND VISUAL CORTICAL CELLS TO STROBOSCOPICALLY ILLUMINATED MOVING LIGHT BARS. G.A. Orban, J. Cremieux*, J. Duysens and H. Maes*. Lab.Neuro- en Psychofysiologie, Katholieke Universiteit te Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

Single unit recordings were made in area 17 and LGN of anesthetized and paralyzed cats. Both for LGN and cortical cells velocity-response curves in opposite directions of motion, response planes and duration-response curves were measured using computer driven stimuli and a randomized multihistogram technique (Orban et al., J. Neurophysiol., 45:1043, 1981; Duysens et al., Brain Res., 231:279, 293, 1982). The most sensitive region of the RF was taken as the middle of the movement path of a stroboscopically illuminated moving light bar. Stimulation was arranged in such way that a flash always fell in the middle of the movement path. A test with 10 velocities and 6 frequencies (50, 29, 15, 8, 4, 2 Hz) shows the changes in velocity characteristics and directionality with strobe frequency. For some cells, we measured the critical fusion frequency (CFF) with a test with 12 frequencies and 2 velocities (the best low velocity and a high velocity). Inspection of the PSTHs in response to stroboscopically illuminated moving light bars allowed us to classify the cells in two groups : a) cells responding to single flashes, b) cells responding only when there are several flashes falling in the field.

The first type was found both in the cortex and LGN and had periodically modulated responses at frequencies up to 15 Hz. These cells had an optimum response for an intermediate frequency (between 4 to 15 Hz), often showing a decrease in response when fusion occurred. At low frequencies the apparent size of the RF was enlarged. All LGN cells belonged to this type which in the cortex was chiefly related to cell types other than S family.

The second type which was exclusively found in the cortex, had no periodically modulated response and showed a decrease of response with decreasing frequency and increasing velocity. This type belonged mostly to the S family, showed strong preference for low velocities and had a duration threshold (Duysens et al., this volume).

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192.13 TWO DISTINCT COMPONENTS OF THE HUMAN VISUAL EVOKED RESPONSE. Erich E. Sutter. Smith-Kettlewell Institute of Visual Sciences, San Francisco, CA 94115.

The human pattern evoked response was analyzed by means of a new technique of nonlinear systems analysis (Sutter, 1982). The contrast reversal of gratings was modulated with specially designed binary sequences and the second order response characteristic was extracted by means of cross-correlation. This approach has the advantage over a more conventional analysis of the pattern response that it permits the identification of cascading subsystems if the nonlinearyty is of zero memory type. On the basis of this assumption two functionally independent components have been isolated which can be compared in their characteristics to the well known response properties of visual cortical neurons in animals.

The subjects were stimulated with reversing gratings (3c/deg) covering areas of the visual field extending from 2^{o} to 10^{o}. The second order nonlinear analysis suggests a cascade of three subsystems: A linear filter at the input stage, a nonlinearity with negligible memory followed by another linear filter at the output stage. On the basis of this model two components can be differentiated by means of the properties of these subsystems and their dependence on the stimulated retinal area:

- Component 1 shows more transient behaviour at the input stage than component 2.
- Both components show similar response structure of the output stage. The response latency of Component 1, however is 20ms shorter than that of component 2.
- Component 1 dominates the large field response whereas both components are well represented when the stimulus is restricted to the foveal and peri-foveal area.

Reference: E.E. Sutter, "A non-stochastic technique for nonlinear systems analysis in the time domain", IEEE Transactions, in press.

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193.1 LACK OF DORSAL LATERAL GENICULATE INPUT TO EXTRASTRIATE CORTICAL AREAS MT AND DORSAL VISUAL 2 IN THE MACAQUE MONKEY. Gregg P. Standage and Louis A. Benevento. P.O. Box 6998, University of Illinois College of Medicine, Chicago, Ill. 60680.

Our recent anterograde autoradiographic tracing studies (e.g, J. Comp. Neurol. 203: 455, 1981) on the projections of the dorsal lateral geniculate nucleus (DLG) to extrastriate (or prestriate) visual associaton cortex have indicated that there are certain functional cortical areas which do not receive DLG input. In order to further clarify the organization of these geniculo-prestriate pathways we performed a series of retrograde tracing experiments with the use of the particularly sensitive wheat germ conjugated horseradish peroxidase (W-HRP). 0.015 µl injections were made in a number of extrastriate areas. Identification of the particular area injected was made by single unit recordings and postmortem histology comprised of both axon and nissl stains and by the presence of retrograde labelled cells in certain layers of striate cortex. The limits of the DLG projection to extrastriate cortex as described in the anterograde experiments seemed to be confirmed. W-HRP filled cells were found in both the DLG and pulvinar when the injections were confined to area 19 and anterior area 18 located on the visible surfaces of the preoccipital gyrus. When the injection was confined to MT (or the movement area of the superior temporal sulcus), no labelled cells were found in the DLG despite a dense number in the pulvinar. In particular it was a crescent shaped region of the dorsal portion of the inferior pulvinar (PI) which contained a dense number of W-HRP filled cells. This region of PI forms a special subdivision which extends into the adjacent,overlying lateral pulvinar. The connections between this region of the pulvinar and MT are reciprocal. Thus, MT would receive its main subcortical input from this specialized zone in the pulvinar and none from the DLG. The present experiments further show that the dorsal portion of visual area 2, in the posteror bank of the lunate sulcus, does not receive a DLG input. This is in contrast to more ventral portions of visual area 2 indicating that visual 2 may be organized differently throughout its extent. When the present results are taken together with those from the anterograde experiments and compared to the findings in the cat, it seems that there are differences in the DLG and extrageniculate thalamocortical organization in the two species. This includes the types of information which are carried by the geniculo-extrastriate pathways, which in the monkey seem to arise, in large part, from the superior colliculus and cortex. (Supported by N.I.H. Grant EY 2940).

193.2 TOPOGRAPHICAL ORGANIZATION OF THALAMIC PROJECTIONS TO PRESTRIATE VISUAL CORTICAL AREAS IN THE MARMOSET (C. JACCHUS). N.L. Hayes and O.-D. Creutzfeldt*. Dept. of Anatomy, Univ. of Mississippi Medical Center, Jackson, MS 39216 and Dept. of Neurobiology, Max Planck Institute for Biophysical Chemistry, Gottingen, FRG.

The visually responsive cortical area anterior to VII in new world primates (VIII) has been subdivided into at least three regions which differ to a large extent in their interconnections with the thalamus in general and with the subnuclei of the pulvinar in particular (Lin and Kaas, 1980). The extent to which thalamic neurons with projections to different regions of VIII are topographically segregated was investigated in Callithrix with a double retrograde tracer study using HRP and ^{3}H-apo-HRP as simultaneously distinguishable tracers. In the same preparations it was possible 1) to explore the possible existence of thalamic neurons with collateral projections to more than one subdivision of VIII, 2) to examine the topographical relationship between populations of thalamic neurons projecting to different areas of VIII, and 3) to explore the pattern by which the three dimensional thalamic nuclei are mapped onto the two dimensional cortical layer IV within and across cytoarchitectonic boundaries.

In each animal, pairs of injections (one HRP, one ^{3}H-apo-HRP) were made in each hemisphere, according to two experimental designs: 1) to combine injections in different subdivisions of VIII and 2) to generate a series of injections along variously oriented lines along the cortex (e.g., parallel to the superior temporal sulcus, parallel to the 17/18 border).

Cortical injections involving layer IV labeled populations of thalamic neurons which in the pulvinar were arranged in dorsomedial to ventrolaterally oriented "slabs" which extended uninterrupted across the borders between the medial, lateral and inferior subdivisions of the pulvinar. In general, a progression from medio-dorsal to ventro-lateral of injection sites on the cortex was paralleled by a progression from rostral to caudal of labeled neurons in the pulvinar-LP complex; a similar progression from anterior to posterior on the cortex was paralleled by a progression from medial to lateral in the thalamus. Three-dimensional reconstruction revealed that the shape of the slabs is quite irregular and that slabs labeled from different cortical areas are closely apposed and interdigitate; however, in no case were double-labeled neurons observed in the pulvinar-LP complex. In the intralaminar nuclei, cells labeled from different cortical injection sites were intermingled with no apparent topographical segregation; among this population several double-labeled cells were observed.

193.3 NORADRENERGIC INNERVATION OF VISUAL SYSTEM STRUCTURES IN MONKEY. J.H. Morrison, S.L. Foote, and F.E. Bloom. The Salk Institute, La Jolla, CA 92037.

A remarkably large proportion of the primate cortex may be considered visual in function. The various visual cortices have been sub-divided and characterized by their cytoarchitectonic properties, patterns of connectivity with thalamic nuclei and other cortical regions, and the visual maps and response properties of their resident neurons. Two major visual thalamo-cortical systems exist: 1) a geniculo-striate projection which terminates heavily in layer IV of primary visual cortex (area 17), and 2) a pulvinar-extrastriate projection which terminates more widely in areas 18 and 19 in the occipital lobe and several visual regions in the inferior temporal gyrus. Given the proposed involvement of the noradrenergic system in cortical sensory processes and attentional mechanisms, it was of interest to determine whether the NA innervation of the pulvinar-extrastriate system differed fundamentally from that of the geniculo-striate system. Using antisera directed against human dopamine ß-hydroxylase, we have characterized the noradrenergic innervation of several of these cortical and sub-cortical visual areas in the squirrel monkey. We recently reported that the noradrenergic innervation of primary visual cortex (area 17) is sparse and exhibits a unique laminar pattern of innervation: layer IV contains very few noradrenrgic fibers, and yet is densely innervated by serotonergic fibers (Morrison et al, PNAS, 1982). Precisely at the 17-18 border, this laminar pattern shifts such that layer IV contains more NA fibers and fewer serotonergic fibers in area 18 than in 17 and the overall density of NA fibers is higher in area 18 than in 17. The pattern of noradrenergic innervation within the visual thalamic nuclei is consistent with that in the visual cortex: the pulvinar is densely innervated by NA fibers, whereas the lateral geniculate nucleus is virtually devoid of NA innervation.

Thus, it appears that in squirrel monkey the pulvinar-extrastriate system is more densely innervated than the geniculo-striate system. The lack of NA innervation of lateral geniculate in the primate is of particular importance given that this thalamic nucleus receives a moderately dense NA innervation in the rat. These and other observations (Morrison et al, Brain Research Bulletin, 1982) suggest that coincident with the extensive phylogenetic development and differentiation of neocortex in the primate there is a parallel elaboration and differentiation of the ascending noradrenergic projection. Supported by USPHS NS 16209, and Training Grant AA 07273.

193.4 CORTICAL PROJECTIONS OF AREA MT IN THE MACAQUE. Leslie G. Ungerleider, Robert Desimone and Mortimer Mishkin. Lab. of Neuropsychology, NIMH, Bethesda, MD 20205.

Area MT is a visuotopically organized area within the caudal superior temporal sulcus characterized by heavy myelination and a high proportion of directionally selective cells. Although it is known that MT receives direct input from both striate cortex and V2, the cortical projections of MT are unknown. To examine these projections and their topographic organization, tritiated amino acids were injected into selected MT sites in five cynomolgus monkeys, and the brains were processed for autoradiography. Visual field representations of the injection sites, which were identified electrophysiologically, included the center of gaze and positions ranging from 8° to 25° in the upper and lower visual fields.

MT was found to project to at least two zones within the superior temporal sulcus (STS) and two in the intraparietal sulcus (IPS), each zone having a distinctive myeloarchitecture. Within STS, one zone is located in the posterior bank of the sulcus, bordering the lower visual field representation of MT. Only MT sites representing the lower visual field and center of gaze project to this zone. As in MT, the central visual field is represented ventrally, and the peripheral field, dorsally.

A second projection zone in STS is a crescent-shaped area in the floor and anterior bank of the sulcus, which surrounds MT anteriorly and medially and lies partly within cytoarchitectonic area PG. Projections to this zone are highly convergent, with only a suggestion of topography. Although the zone is myeloarchitecturally uniform, a separate, discrete patch of label consistently seen at the posteromedial tip of the crescent suggests that this segment may be a separate projection area.

Within IPS, a posterior projection zone begins in the annectent gyrus caudally and extends along the fundus of the posterior third of IPS. We previously found a direct striate projection to this zone, which may correspond to Zeki's area V3A. The other zone in IPS lies in the anterior two-thirds of the sulcus, extending from the fundus onto the posterior bank; this zone is located in area PG. Both projection zones, anterior and posterior, receive convergent input from all parts of MT, including upper and lower visual field representations.

In addition to the forward projections of MT, which span all cortical layers and often appear as columns, there are backward projections to striate cortex and V2. Projections to both areas are laminar, terminating in layers I and VI and also in IVa in striate cortex.

The results suggest that MT provides a major link through which visual information is relayed forward from striate and prestriate areas into the parietal lobe.

193.5 ANTAGONISTIC DIRECTION SPECIFIC MECHANISMS IN AREA MT IN THE OWL MONKEY. Francis Miezin*, EveLynn McGuinness* and John Allman. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

It is well known that most Area MT neurons are strongly selective for the direction of stimulus motion. We wished to know how MT neurons behaved when stimuli were presented against moving textured backgrounds such as occur commonly in natural stimulus conditions. We have explored the receptive field structure and directionally selective mechanisms in MT using a newly developed microprocessor-based video display that can present simultaneously bars and random dot patterns moving in different directions. In nearly all MT neurons tested, when a bar moving in the cell's preferred direction is presented against a background of random dots moving in the same direction, the response is greatly reduced relative to the response to the bar moving on a background of stationary random dots. When the background is moved in the opposite direction, the response to bar stimulation in the preferred direction is either less inhibited or in some cases strongly facilitated. The effects of background stimulation generally could be obtained by stimuli restricted to the neuron's conventionally defined receptive field but were stronger when background stimulation extended into the surrounding visual field.

In a related set of experiments we sought to map the extent and nature of the antagonistic mechanism in the surround. By continuously driving MT neurons by stimulating the conventionally defined receptive field and by simultaneously moving fields of random dots in the surrounding visual field, we have found that MT neurons are affected by stimuli presented at considerable distances from the conventional receptive field. These surround effects are often directionally selective with the preferred direction of the center being the direction of maxiumum inhibition in the surround. This antagonistic direction-selective mechanism provides an ongoing comparison between movement occurring locally in the visual field and more global retinal image movements such as would occur during eye and head movement or bodily transport. Thus they may be related to the mechanisms that accomplish the perceptual stability of the visual world during eye, head and body movements as well as to perceptual mechanisms in figure-ground discrimination. (Supported by NSF grant BNS-77-15605, NIH grant EY03851, the Pew Memorial Trust and the L. S. B. Leakey Foundation.)

193.6 INDICATIONS OF FUNCTIONAL RELATIONSHIP BETWEEN PREFRONTAL AND INFEROTEMPORAL CORTEX. J.M. Fuster, J.P. Jervey* and R.H. Bauer. Dept. of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

In the monkey, local cooling of either prefrontal or inferotemporal cortex--but not parietal cortex--induces a deficit in performance of a visual memory task (delayed matching to sample). During performance of that task, prefrontal and inferotemporal cells have been seen to undergo characteristic changes of activity correlated with the presence and short-term retention of the memorandum (a color). Anatomical studies demonstrate reciprocal connections between prefrontal and inferotemporal areas. The present study explores the possible functional significance of that reciprocal connectivity in visual memory performance.

Rhesus monkeys were trained in delayed matching to sample with two colors, red and green. One of the colors, the sample, was presented at the start of each trial; after a delay (16-20 sec), both colors were presented side by side and the animal had to choose the one matching the sample. The sample color and its location at the time of choice were changed randomly between trials. In some of the animals, bilateral cooling probes were implanted on dorsolateral prefrontal cortex, covering the sulcus principalis and the area directly above and below; micropositioner pedestals were implanted over inferotemporal cortex. The other animals were prepared in the reverse manner: with cooling probes over a large portion of the inferotemporal convexity and micropositioner pedestals over prefrontal cortex.

Single-unit discharge was recorded with microelectrodes during task performance. Prefrontal cooling was tested on 60 inferotemporal units (most in middle temporal gyrus and lower bank of superior temporal sulcus) and inferotemporal cooling was tested on 49 prefrontal units (most in inferior convexity and lower bank of sulcus principalis). The bilateral cooling of either cortical region induced the following changes in the other: 1. Changes in the spontaneous firing of many units; increases of firing predominated over decreases. 2. Both augmentations and diminutions of unit reaction to the sample color. 3. Attenuation of the differences in the reaction of some units to the two sample colors. 4. Attenuation of some sample-related differences in unit activity during the retention period. In no case were color-dependent differences increased by cooling.

The results indicate that the two cortical regions investigated are normally subject to mutual influences, probably by way of the reciprocal connections that link them. The effects of cooling on performance and on unit activity suggest that those mutual influences subserve visual discrimination and short-term memory.

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193.7 A NEW VISUAL AREA IN THE PARIETO-OCCIPITAL SULCUS OF THE MACAQUE. E. Covey, Div. Otolaryngology, Duke Univ. Med. Ctr., Durham, N.C. 27710, R. Gattass*, Dept. Neurobiologia, Instituto de Biofisicia, UFRJ, Rio de Janeiro RJ, Brazil and C.G. Gross, Dept. Psychology, Princeton Univ., Princeton, NJ 08540.

We report a previously undescribed cortical visual area in the macaque which we provisionally call Area PO. It lies on the anterior bank of the parieto-occipital sulcus and adjacent portions of the precuneate gyrus and the anterior bank of the intraparietal sulcus. Posteriorly and medially, Area PO borders Area V2 along the floor of the parieto-occipital sulcus. Dorsally and anteriorly, it borders Area 7. The ventrolateral border of Area PO lies in the floor of the intraparietal sulcus, perhaps adjacent to Area 3A of Zeki. Area PO is distinguishable from each of the surrounding areas in fiber stained sections and in neuronal response properties such as receptive field size. On the basis of anatomical studies of Ungerleider et al., it does not appear to receive projections from either V1 or V2.

The representation of the visual field in Area PO was mapped with multi-unit electrodes in five M. fascicularis. The animals were immobilized and anesthetized and in each animal 20-40 electrode penetrations were typically made over several recording sessions. Area PO contains a representation of the entire contralateral visual field. Its visuotopic organization appears more complex than that of visual areas V1, V2 or MT. The representation of the upper field is located medially and that of the lower field is located laterally. The representation of the horizontal meridian lies along the ventromedial border of Area PO (within the parieto-occipital sulcus) and splits to extend up the anterior bank of the parieto-occipital sulcus. One portion of the representation of the vertical meridian lies along the ventrolateral border (within the intraparietal sulcus). Another portion lies along the dorsal and anterior border (within the parieto-occipital sulcus and on the medial surface). The representation of the periphery of the visual field is relatively large as compared to that in V1, V2 or MT. This finding and the medial location of Area PO suggests that it may be homologous to Area M in the owl monkey described by Allman and Kaas.

193.8 A PHYSIOLOGICAL COMPARISON OF THE LATERAL PULVINAR AND AREA 7 IN THE BEHAVING MACAQUE. Steven E. Petersen*, David Lee Robinson, and William Keys*. Lab. Sensorimotor Research, National Eye Inst., Bethesda, MD 20205.

Anatomical data show that a thalamocortical pathway exists between a subdivision of the lateral pulvinar and area 7 of the cerebral cortex. We recorded single cells from awake, trained rhesus monkeys, and compared the functional properties of cells in area 7 and the region of the lateral pulvinar reported to project to area 7. The neurons of these two areas share several passive visual properties:

(1) crude, if any, retinotopic organization.
(2) relatively long mean latencies (pulvinar = 88 ms; area 7 = 96 ms).
(3) very broad latency ranges (pulvinar = 36-224 ms; area 7 = 40-236 ms).
(4) large receptive fields.
(5) a lack of orientation selectivity.
(6) a preference for large stimuli, although frequently the receptive fields in the pulvinar have antagonistic surrounds or internal inhibition, a property rare for area 7 neurons.
(7) a large number of pan-directional cells, although there is a higher incidence of directional selectivity in our sample of lateral pulvinar cells than in area 7 cells.

About one-third of the visually-responsive cells in the lateral pulvinar, and one-half of the area 7 cells have an enhanced response when the discharge to a stimulus which is the target for a saccadic eye movement is compared to the activity when fixation is maintained. We have not determined if these cells ot the lateral pulvinar also give an enhanced response when the animal attends to a peripheral stimulus but does not saccade to the light, a characteristic of many cells in area 7.

Some cells in the lateral pulvinar discharge in relation to saccadic eye movements. These cells respond during and/or after eye movements with excitatory, inhibitory or combined inhibitory-excitatory responses. Such activity is frequently present with saccadic eye movements made spontaneously in total darkness. We have not identified cells in area 7 that are active with spontaneous saccades nor are there published reports of such activity.

Our data suggest that the ascending pathway from this region of the lateral pulvinar may be an important source of visual input to area 7. However, these experiments imply that the non-visual activity present in the lateral pulvinar probably influences other areas of the brain.

193.9 PULVINAR INFLUENCES ON GENICULO-CORTICAL ACTIVITY IN THE CAT. A. L. Sica and W. S. Battersby*. Lab. of Neurophysiology, Queens College, CUNY, NY,NY 10467. The effects of pulvinar stimulation on the response in area 17 to a shock in the lateral geniculate nucleus were investigated with both gross and microelectrodes. Data were obtained from 27 male cats prepared for acute study, and maintained on a mixture of N_2O and O_2 during experimentation. The gross recordings show that activation of either the ipsilateral or contralateral pulvinar increases the amplitude of the cortical response; the magnitude and time course of this response enhancement were similar following stimulation of either pulvinar. These effects were abolished by a "functional block" (topical application of KCl) of the medial to posterior-medial suprasylvian gyrus. Extracellular recordings in area 17 show that stimulation of the ipsilateral pulvinar influences the suppression period and afterdischarge of the unit response to a geniculate shock. These effects varied as a function of interstimulus interval; i.e., suppression was shortened and the afterdischarge increased at brief delays, in contrast, suppression was prolonged and the afterdischarge decreased at longer delays. The close similarity of these effects with those found with stimulation of the midbrain reticular formation (Alter & Battersby, 1973) suggests that the pulvinar and suprasylvian gyrus may form a thalamo-cortical pathway for relaying activity ascending bilaterally from midbrain structures to striate cortex. Recordings of pulvinar units revealed both functional afferents from the contralateral superior colliculus, and the presence of inhibitory influences descending from the suprasylvian gyrus. In conclusion, our results support a relay function for the pulvinar, and suggest that it may participate in the extensive changes in cortical activity, e.g., arousal, observed with midbrain stimulation.

Alter, I., and Battersby, W. S. Temporal dispersion of unit discharges in visual cortex due to MRF-LGB interaction. The Physiologist, 1973, 16 (3),

193.10 CHANGES IN THE VISUAL EVOKED RESPONSE FOLLOWING Ep ABLATION. R.J. Sinclair* and S. Horenstein. Dept. of Neurology, Saint Louis University, Saint Louis, Missouri 63104.

Changes in the early portions of the visual evoked response (VER) and some later components occurred after unilateral ablation of the cortex of the posterior ectosylvian region in cats trained in a visual discrimination task.

Five adult cats had been trained in a Y-maze equipped with mechanical dippers to deliver food reinforcement and photostimulators to deliver an intense short duration flash. The Y-maze consisted of three long alleys connected by a central atrium. The mechanical dippers were placed at the end of the alley opposite the atrium. The animals were trained to leave the alley where they had just obtained food and move to either of the remaining two for the next reinforcement. Silver ball electrodes were implanted subdurally over both suprasylvian visual areas (Vss), with a frontal sinus reference electrode. EEG signals were delivered by telemetry to an FM receiver and then fed into an electroencephalograph connected to a PDP11 computer for averaging. During the preoperative testing period, evoked responses (ER) for 512 milliseconds were obtained following left and right unilateral and bilateral and homologous double simultaneous stimulation (DSS). Three experimental animals then received unilateral lesions of area Ep. One of the controls received an unilateral AI lesion and the other one of AII. Postoperative testing repeated the preoperative procedure.

Pre- and post-operative data were analyzed statistically for latency and amplitude changes in the major components of the ER for two groups: two Ep subjects with neglect, and two AI/AII subjects without neglect. The third Ep subject had ipsilateral Vss degeneration under the electrode site and no neglect, and was excluded from analysis. The data disclosed different patterns of change for the neglect compared to the non-neglect subjects, suggesting that those associated with neglect resulted specifically from the lesion of area Ep. In the neglect group the amplitude of the earliest ER component was affected. This possibly represents the first stimulus registration within the cortex. Since this followed a cortical lesion, a corticofugal mechanism which modulates incoming sensory information at lower levels of the sensory pathways is inferred. Significant changes were found in the latency of early components Po/No, conventionally associated with a comparator process for identifying novel or motivationally significant stimuli. Changes in latency of some later components may indicate a possible "chain reaction" following from disturbances in early stages of sensory processing.

193.11 EXTRAPOLATION OF MOTION PATH IN HUMAN VISUAL PERCEPTION V.S. Ramachandran* and S.M. Anstis* (SPON: J. Brockes) School of Social Sciences, University of California, Irvine, CA 92717

Moving objects have two properties. First, they persevere in their state of uniform motion along a straight line (Newton's first law). Second, all points on the surface of a moving object tend to move in the same direction with identical velocities.

Since the visual system has evolved to process information from the physical world, one might expect similar principles for the perception of moving objects. Using dynamic, ambiguous apparent motion displays we have found that a point which moves in one direction will tend to be perceived as continuing in its motion in that direction.

We used a matrix of four dots forming the four corners of a diamond subtending 1°. Pairs at opposite corners flashed in alternation (SOA=100 msec) gave rise to ambiguous motion that fluctuated between two oblique directions, either NW-SE or NE-SW. If the four dots were embedded in two long parallel oblique rows of dots flashed in apparent motion sequence, a "visual momentum" effect strongly favoured the perception of motion along this oblique direction--i.e., collinear to the flashing sequence. This might be regarded as a perceptual equivalent of Newton's law.

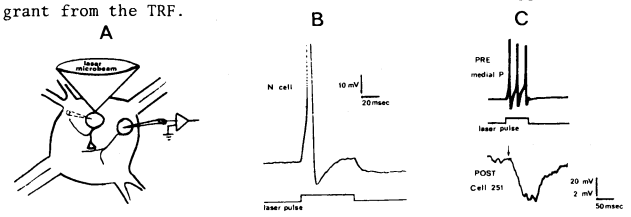
In our second experiment we alternated two uncorrelated random-dot patterns (subtending 5°) in a continuous cycle (SOA=75 msec). Surprisingly, we did not see random incoherent motion. For any given small area the dots tended to move in identical directions giving rise to 'cohesive motion'; but the actual direction fluctuated from moment to moment. To one margin of the display we then added a vertical strip of dots 1° wide which was correlated in alternate frames and moved left-right unambiguously. The entire display now "adhered" to the strip and moved synchronously with it. We conclude that when viewing ambiguous dynamic noise there is a tendency to see spatially uniform motion.

The results of these two experiments are complementary and they suggest that there are at least two properties of moving physical objects that translate into specific rules which are incorporated into the visual system. One of these is the fact that moving objects usually don't change direction abruptly. The second rule is that when a surface moves all points on it generally move in perfect synchrony. These redundancies of moving objects in nature are captured by the brain's motion detecting networks and manifest as a spontaneous tendency for seeing uniform unidirectional motion when confronted with ambiguous dynamic dot displays.

It would be interesting to look for physiological correlates of these contextual effects both in striate and extrastriate areas where direction specific cells are found.

194.1 A NOVEL TOOL FOR EVALUATION OF NEURONAL NETWORKS: LOCALIZATION OF PRESYNAPTIC NEURONS BY LASER PHOTOSTIMULATION OF INVERTEBRATE CNS NEURONS. I.C. Farber* and A. Grinvald. (SPON: V.I. Teichberg).Dept. of Neurobiology, Weizmann Inst. of Science, Rehovot, Israel.

A microelectrode search for the neurons which are presynaptically connected to a given cell may be difficult even in a simple central nervous system if many pairs of cells have to be impaled, or if the neurons are small, or for other technical reasons. An attractive solution is to be able to stimulate all presynaptic neurons with laser light; thus a rapid scanning of a laser microbeam over each of the individual neurons may be used to find the neurons which will synaptically affect a given electrically-monitored neuron. We discovered a photostimulation probe designated RGA-30. After the dye molecules bind to neuronal membrane, illumination of a stained neuron with a laser microbeam induces a rapid depolarization and subsequent firing which activates its synaptic endings. Our experiments were carried out on the segmental ganglia of the leech Hirudo medicinalis and on the supraesophageal ganglion of the giant barnacle. The ganglion was stained with a normal saline containing 20 uM of the probe (a thiobarbituric acid pyrazolone oxonol dye) for 20 min. A 50 mW He/Ne laser was used with appropriate optics to form a 1 u spot of light. This microbeam could be positioned precisely on the desired membrane spot by adjusting the microscope focus under visual control. Fig. 1A illustrates the experimental arrangement. Figure 1B shows the instantaneous depolarization of the N sensory cell with a pulse of light. This cell could be restimulated 7 times. Figure 1C shows that the medial sensory P cell which was photostimulated (top two traces) is indeed presynaptically connected to cell 251 by an inhibitory synapse. Using this dye it was possible to photostimulate all the 14 sensory cells, the Retzius cells and 10 different pairs of motoneurons. In each case the membrane potential quickly recovered within 1-5 mV, but the membrane resistance dropped to a value of 70-50% of the original. Preliminary TTX and ionic substitution experiments suggest that a significant part of the depolarization is due to light activation of the sodium channels. Supported by a grant from the TRF.



194.2 TWO PHASES OF SPIKE FREQUENCY ADAPTATION IN APLYSIA NEURONS HAVE DIFFERENT SENSITIVITY TO CALCIUM INFLUX. D. V. Lewis* and W. A. Wilson. (SPON: W. Anderson) Neurophysiology Lab, Dept. of Pediatrics, Duke Univ. Med. Sch., Durham, N.C. 27710.

When the silent cell, R2, of Aplysia californica is depolarized by a 60 sec constant current stimulus, two phases of spike frequency adaptation are observed. There is an initial rapid phase lasting from 5 to 10 sec during which the firing frequency falls to between 10 and 40% of the initial rate, followed by a slow adaptation phase characterized by a very gradual steady decline in firing rate (Zbicz, K. and Wilson, W. A. J. Pharmacol. Exp. Ther. 217:222-227, 1981). When intracellular free calcium is monitored by Arsenaso III spectrophotometry, calcium rises abruptly during the first 10 sec of the 60 sec spike train and then shows little change during the remaining 50 sec. Thus the most rapid rise in calcium occurs simultaneously with the rapid adaptation phase. Removal of extracellular calcium, intracellular EGTA, or blockade of calcium influx with calcium blockers each reduces or eliminates the fast phase of adaptation. Although intracellular calcium changes are not detectable the firing rate shows a slow steady decline during the entire 60 sec stimulus.

We conclude that the rapid adaptation phase is strongly dependant upon calcium influx and probably mediated by a calcium activated potassium conductance (Lewis, D. V. and Wilson, W. A. J. Neurophysiol. In press). The slow phase of adaptation is far less dependant upon calcium influx although the possibility remains that it may depend upon a intracellular calcium pool not detected by Arsenazo III.

194.3 ENHANCEMENT OF SLOW OUTWARD CURRENT BY PENTOBARBITAL IS INDEPENDENT OF Ca^{++} ENTRY. G.J. Evans*, J.R. Huguenard, W.A. Wilson, and D.V. Lewis* (SPON: A. Roses). V.A. Epilepsy Center and Departments of Pharmacology and Pediatrics, Duke University, Durham, NC 27710.

It has been demonstrated that pentobarbital (PB) enhances slow spike frequency adaptation (SFA) and the slow outward K^+ current (SOC) associated with slow SFA in the Aplysia neurons R2 and LP1. Since Ca is known to enter these cells during a spike train or during a 60 second depolarization in voltage clamp (VC), it was suspected that SOC might represent the Ca activated K^+ conductance (gK_{Ca}) described by Meech et al. (Comp. Biochem. Physiol., 1972, 42: 493). We therefore have examined the relative importance of depolarization vs. Ca entry for SOC and slow SFA. Compartmental analysis of the tail currents that follow a VC depolarization or a spike train consistently reveals two components. Since this analysis is a sensitive index of changes in SOC (Evans, et al. Soc. Neurosci. Abst. 1981: 810), it was used in this study.

100μM PB had no effect on the amplitude or kinetics of tail currents that followed Ca entry accompanied by little or no depolarization (i.e. intracellular Ca injection or a 2 second spike train). This was true even if [Ca]i was elevated for as long as 60 seconds by eliciting 1 controlled spike per second from VC. In addition, the Ca mediated hyperpolarization induced by white light in these cells was unaffected by PB.

When Ca entry was prevented by replacement of [Ca]o with a Ca channel blocker (1mM La) or addition of 3mM EGTA to Ca-free sea water, the tail currents following a short spike train or prolonged slow spiking were abolished, indicating their dependence on Ca entry. PB was unable to restore them. With Ca entry restricted, the SOC and the tail current were reduced, but PB remained effective in enhancing SOC.

These data indicate that PB does not act on the K_{Ca} channel. Also, SOC and its enhancement by PB do not depend on Ca entry. Yet [Ca]i was shown to play a key role in SOC. Intracellular injection of EGTA, in conjunction with 0 Ca - 3mM EGTA sea water, reduced the SOC tail current and abolished the post spike tail current.

It would appear that while SOC is sensitive to the level of [Ca]i, barbiturates are able to enhance SOC without increasing Ca entry or affecting the interaction of the K_{Ca} channel with Ca. We hypothesize that SOC may depend on the activation of K_{Ca} channels by Ca from an alternate source, i.e. the voltage sensitive release of Ca from intracellular stores. Furthermore, we propose that barbiturates enhance SFA and SOC by increasing depolarization induced intracellular Ca release.

Supported by NIH Grant 15212 and the VA Hospital, Durham, NC.

194.4 DIPHENYLBARBITURIC ACID: A SPECIFICALLY ANTICONVULSANT BARBITURATE? J.R. Huguenard and W.A. Wilson, V.A. Epilepsy Center and Departments of Pharmacology and Medicine, Duke University Medical Center, Durham, NC 27705.

Phenobarbital (PB) is widely used as an anticonvulsant agent. Unfortunately, the neurodepressant effects of PB are not totally selective in supressing the excess neuronal activity associated with seizures, and often cause some drowsiness.

Diphenylbarbituric acid (DPB) is a compound structurally similar to two commonly used anticonvulsants, phenobarbital and diphenylhydantoin. DPB has been demonstrated to be effective in preventing seizures induced by pentelenetetrazol and electroshock (Raines, et. al., JPET 186: 315-22, 1973). Although DPB was effective in these anticonvulsant drug screens, it showed relatively little neurotoxicity.

A proposed anticonvulsant mechanism of the barbiturates is the enhancement of a voltage sensitive slow outward current (SOC) which serves to supress repetitive firing during a prolonged stimulation. The supression of repetitive firing with time is termed spike frequency adaptation (SFA). SFA occurs in two phases, an initial rapid decrement in firing rate complete within 10 seconds followed by a much slower phase which occurs over a period of minutes. Barbiturates enhance SOC and the slow phase of SFA (Zbicz and Wilson, JPET 217: 222-27, 1981).

We were interested in determining whether barbiturate enhancement of SOC and adaptation correlated with anticonvulsant effects rather than sedative effects. We therefore wanted to determine whether DPB, a specifically anticonvulsant, non-sedative barbiturate would demonstrate an enhancement of the SOC and adaptation.

Giant neurons from Aplysia californica were used in this study. Prolonged voltage clamp pulses to near spike threshold were used to elicit SOC. The amount of SOC was quantitated by analyzing the tail currents (obtained upon return to holding potential) which could be represented by the sum of two exponential decay processes (components).

DPB enhanced the slow phase of adaptation, the SOC and the amplitude of the slow component of tail. Increasing effects on these parameters were seen in the concentration range of 1 to 20μM. This range is at least an order of magnitude lower than the effective range for phenobarbital.

It appears, therefore, that enhancement of SOC and adaptation by barbiturates is not involved in the mechanism of producing neurotoxicity (drowsiness), but rather that this enhancement may correlate with the anticonvulsant action of these drugs.

Supported by NIH grant 15212 and V.A. Medical Center, Durham, North Carolina.

194.5 SEROTONIN ENHANCES INWARD CURRENT IN APLYSIA, Terry C. Pellmar. Laboratory of Preclinical Studies, NIAAA, Rockville, MD 20852.

Iontophoretic application of serotonin (5HT) to RB cells of Aplysia abdominal ganglion produces a voltage-dependent inward current at potentials more depolarized than -40mV. In a previous study (Pellmar & Carpenter, J. Neurophysiol. 44 1980, 423), ion substitution experiments and application of channel blocking agents suggested calcium as the current-carrying ion. To further test this proposed mechanism, the following series of experiments were performed. In the presence of 30mM tetraethylammonium (TEA), 4mM 4-aminopyridine (4-AP) and 10mM barium (substituted for calcium), RB cells showed a negative slope region (NSR) in their current-voltage (I-V) relationship when measured at the end of 2 second commands from a holding potential of -60mV. Iontophoretic application of serotonin in this solution failed to produce a voltage-dependent response when membrane potential was held at -10mV. Yet, when 5HT ($5x10^{-4}$M) was added to the solution, the NSR was enhanced. When holding potential was depolarized to -10mV, the NSR as well as the effects of serotonin were greatly reduced. Addition of 20mM cobalt to the bathing solution blocked both the NSR and the inward current produced by serotonin. Changes in potassium concentration (1mM, 10mM and 40mM) produced no consistant shift in voltage-sensitivity nor change in amplitude of the current elicited by addition of serotonin. Intracellular injection of cesium broadened action potentials and often greatly reduced the iontophoretically elicited voltage-dependent response in normal sea water. However subsequent addition of TEA, 4-AP and Ba revealed an NSR which could be enhanced by 5HT. These experiments support the conclusion that in RB cells, serotonin produces a voltage-dependent current carried by calcium.

TCP was supported by NSF grant no. PDF 816030.

194.6 SLOW SYNAPTIC ACTIONS PRODUCED BY CELL R15 IN APLYSIA. R.O. Brown*# and E. Mayeri#+. #Dept. Physiology, Univ. of Calif., San Francisco, CA 94143 and +Dept. Basic Sciences, California College of Podiatric Medicine, San Francisco, CA 94115.

Cell R15 is a large, neurosecretory, bursting pacemaker neuron in the abdominal ganglion of Aplysia californica. It is thought to be a peptidergic neuron because it synthesizes large amounts of low molecular weight proteins and polypeptides (Loh and Gainer, Brain Res. 92:193, 1975). Moreover, a pronase-sensitive factor from isolated R15 somata causes rapid weight increase when injected into the hemocele of Aplysia, suggesting that R15 is involved in the regulation of water balance (Kupferman and Weiss, J. Gen. Phys. 67:113, 1976). We report here that R15 has effects on neurons of the left lower quadrant (LLQ) of the abdominal ganglion, producing slow synaptic potentials in phase with R15's bursting pacemaker activity.

R15 was typically firing in 6 second bursts of 25 spikes, with 10 second silent periods between bursts. The most commonly seen effect was an excitation which began a few seconds after the onset of the R15 burst, rose slowly and smoothly to a peak depolarization of as much as 8 mV, and returned slowly to baseline when the R15 burst was over. The magnitude of the depolarization could be reduced in a graded fashion by prematurely terminating the R15 burst with hyperpolarizing current, and it could be increased by depolarizing R15 during its burst. That the response could be manipulated in this way suggests that the interaction is a direct one. The depolarizing response was also reduced when the postsynaptic cell was hyperpolarized.

While R15's effect was most often excitation, in some instances it was inhibition or biphasic excitation-inhibition. In all cases the effects disappeared when R15 was prevented from firing by injection of hyperpolarizing current. When R15 was thus turned off, some active neurons excited by R15 became silent, and some silent neurons inhibited by R15 began spiking.

The R15 actions were not seen in all animals. Often there were no effects on LLQ neurons, even though R15 was firing normally, and appeared to be healthy. When the action was present, however, it affected many or most of the LLQ neurons on the dorsal surface, and persisted for the duration of the experiment (up to 10 hours). This raises interesting questions as to what factors govern this interaction, and where the site of control is located.

Because R15 produces peptides, and considering the slow time course of its synaptic actions, it seems likely that the transmitter mediating R15's effects on LLQ neurons is a peptide.

Supported by NIH Grant NS16490.

194.7 AN ANALYSIS OF THE BURSTING BEHAVIOR OF THE APLYSIA R_{15} NEURON DURING EXPOSURE TO NORMOXIC AND HYPOXIC BATH CONDITIONS. P.E. Coyer. Dept. of Neurol. and the Neurosciences Program, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

For the R_{15} neuron of the Aplysia abdominal ganglion, measurements of membrane potential (E_M, at a point corresponding to the trough of the after-hyperpolarization), the amplitude of the burst pacemaker potential (BPP), the interburst interval, and spike frequency within a burst were carried out during periods of normoxia (control), hypoxia (experimental), and reoxygenation. An analysis of the bursting behavior of R_{15} was performed by plotting the spike frequency per burst versus the amplitude of the BPP and BPP as a function of E_M. Preliminary data for 5 R_{15} neurons is presented below. To test for possible changes in resting gK during hypoxia, R_{15} neurons were exposed to a low (0.1 mM versus the normal 10 mM K^+) concentration during normoxia and hypoxia. Under these conditions, R_{15} hyperpolarized to a greater extent during exposure to hypoxia, and it became more apparent that an increase in the resting conductance to potassium accounted for the hyperpolarization observed during hypoxia. This observation substantiated the finding of an increase in the amplitude of the BPP and a corresponding increase in the spike frequency per burst. In fact, the spike frequency per burst was a linear function of the height of BPP, which increased during hypoxia probably due to hyperpolarization of the membrane potential. The length of the interburst interval was neither affected by the degree of membrane hyperpolarization nor the change in height of the BPP. These changes in E_M, BPP, and spike frequency per burst were reversible following reoxygenation, but this observation does not exclude the possibility of a synaptic mechanism which increased resting gK.

	# of cells	$\bar{X} \pm$ S.D. E_M (mV)	BPP (mV)	SPIKES (#)
Normoxia	5	-37.3 ± 2.8	3.1 ± 0.5	4.8 ± 0.6
Hypoxia	5	-48.9 ± 3.6	7.9 ± 0.3	10.5 ± 2.1
Reoxygenation	5	-39.8 ± 1.6	4.1 ± 0.1	6.2 ± 1.2

194.8 INTRACELLULAR FREE POTASSIUM CONCENTRATION IN SNAIL NEURONES MEASURED WITH ION-SELECTIVE MICROELECTRODES. F.J. Alvarez-Leefmans and S.M. Gamiño*. Department of Neuroscience, CINVESTAV del IPN, Apartado Postal 14-740, México 14, D.F.

Many studies on intracellular ionic activities in neurones have been made in the soma of cell 1F of Helix ganglia and yet very few measurements have been reported on the intracellular free potasium concentration $[K^+]_i$. Since K^+ is by far the most abundant cytosolic cation, precise knowledge of its free intracellular concentration is of great importance not only for understanding several membrane phenomena but also for correct and realistic calibration of ion-selective microelectrodes for measurement of other intracellular ions. $[K^+]_i$ was measured in cell 1F and 2F of the sub-oesophageal ganglia of Helix aspersa, using K^+-selective microelectrodes prepared with Corning liquid ion-exchanger 477317 by a method similar to that described by Tsien and Rink (J. Neurosci. Methods., 4: 73, 1981). The electrodes had a virtually ideal, Nernstian, response between 70 and 110 mM K^+ in mixed calibration solutions whose composition was such that the sum of $[Na^+]$ and $[K^+]$ was constant (110 mM) and buffered (pH=7.5) with 5 mM Na-Hepes. Adding 3 mM Mg^{2+} to the solutions had no measurable effect on electrode response.

The ganglia were superfused with a solution containing (mM): NaCl, 80 or 100; KCl, 4; $MgSO_4$ or $MgCl_2$, 5: $CaCl_2$, 4.5; Na-Hepes, 5; pH 7.5 equilibrated with 95% O_2 and 5% CO_2. All experiments were done at room temperature (20-24.5°C), during February and March 1982. Cells 1F and 2F of the right parietal ganglia were impaled first with a 3 M KCl microelectrode (5-20MΩ) and then the K^+-selective microelectrode. Four experiments were selected which satisfied the following criteria: (1) resting potential more negative than -47mV, and not,or only transiently perturbed by the second impalement, (2) K^+-electrode gave Nernstian responses between 70 and 110 mM before and after impalement. For these neurones the mean intracellular K^+ activity corresponded to the activity of 88.1 mM free K^+ concentration in the calibrating solutions (S.E. ± 2.2 mM, range 84-93.2 mM). Other impalements which did not meet the above criteria but allowed a measure of $[K^+]_i$ to be made, in cells with resting potentials more negarive than -38 mV gave a mean $[K^+]_i$ of 93.8 mM (S.E. ± 3.6 mM, range 85-102.4 mM, n=4).The $[K^+]_i$ in these cells was not significantly ($P > 0.3$) different to that found in the first group. The mean $[K^+]_i$ for both populations of cells was 91.0 mM (S.E. ± 2.2).

194.9 MOTORNEURON MEMBRANE CONSTANTS AND SIGNALING PROPERTIES IN THE NEMATODE ASCARIS. R.E. Davis* and A.O.W. Stretton. Neurosciences Program and Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706.

In the motornervous system of Ascaris, the dorsal and ventral cords are connected by a repeating pattern of single identified motorneuron processes, called commissures (Stretton, A.O.W. et.al., PNAS, 75: 3493. 1978). Using two intracellular microelectrodes for stimulation and recording, we have determined the cable properties of these commissural motorneurons:

Property	Average	Range
R_{input}(MΩ)	6	4.8-10
λ(mm)	6.3	5-11
R_i (Ωcm)	82	49-128
R_m (Ωcm^2)	53,000	34,000-108,000
τ_m (ms)	35	25-48
C_m (μF/cm^2)	.83	.5-1.2

The membrane capacitance (C_m) is similar to that found in other biological membranes. Due to the large membrane resistance (R_m), however, the space constant (λ) is relatively high, accounting for the observed ability of these cells to conduct spontaneous passive signals over long commissural distances with only slight decrement. The only signals we have been able to evoke in these motorneurons are graded signals, i.e., signals which lack a clear threshold and whose amplitude is a graded function of stimulus strength. In these cells we have never observed spontaneous all-or-none action potentials nor have we been able to evoke them (at normal resting potential or after hyperpolarization). Thus, lacking the classical long-distance signaling mechanism, these motorneurons rely on their unusual membrane properties to convey information effectively over the long distances separating the ventral cord (input zone) and the dorsal cord (output zone to muscle). Nonetheless, active voltage-dependent membrane channels do appear to be present as indicated by our ability to elicit anode-break responses. These responses, however, are themselves graded (i.e., increase in amplitude with increasing strength of the hyperpolarizing pulse).

We have also examined neuromuscular synaptic transmission. For both exitatory and inhibitory motorneurons, synaptic transmission is graded. In addition, we have evidence that these cells tonically release neurotransmitter. Taken together, these cable and motorneuron signaling properties seem appropriate for a system in which passive signaling plays a major role. (Supported by USPHS Grant # AI 15429.)

194.10 POSTSYNAPTIC HABITUATION IN IDENTIFIED FLATWORM NEURONES? C. L. Keenan* and H. Koopowitz (SPON: R.K. Josephson). Developmental and Cell Biology, University of California, Irvine, CA 92717.

Multimodal sensory cell types (bBRA and SC) in the brain of the polyclad flatworm, Notoplana acticola, habituate to repeated light, vibration and touch stimuli. The rates of habituation depend on a number of parameters, including stimulus intensity, frequency and temporal pairing of different modalities. Although latency decreases with increasing stimulus intensity, it does not change significantly during habituation. Habituation is accompanied by decreases in epsp amplitude. This could either be due to decreases in presynaptic transmitter release or decreased postsynaptic efficacy. We have investigated changes in postsynaptic membrane properties following habituation and dishabituation. Membrane properties were evaluated by analyzing the charging characteristics of cells during the passage of square-wave hyperpolarizing current pulses. Using Rall analyses (Rall, 1969) we find decreases in membrane resistance following habituation to vibration stimuli. Dishabituation by light-off stimuli, following a train of vibration stimuli, is accompanied by an increase in membrane resistance. Both the time constant and electronic length also show concommitant changes that suggest altered membrane resistivity during habituation and dishabituation.

The response of the membrane to subthreshold depolarizing pulses before and after habituation are contrasted with the previous data. Changes in post synaptic cell membrane characteristics during habituation will be discussed.

(This work was supported by grant NS13713-05 from NIH)

194.11 ESTIMATION OF SPECIFIC MEMBRANE AND CABLE PROPERTIES OF LEECH RETZIUS CELL ELECTROTONIC COUPLING. K.M. Chapman and J. Yang* Neurosciences Section, Brown Univ., Providence, RI 02912

The paired Retzius (R) cells within the leech segmental ganglia are coupled by a non-rectifying electrontonic junction, which has been described as a simple resistance between the two somata. However, several investigations of the coupling permeability, using tracer molecules, suggest the electrical contacts occur between overlapping fine axonal arborizations deep within the neuropil. If so, the electrotonic coupling may in fact show cable-like properties. The present investigation tests this possibility, and also provides new quantitative estimates of the passive electrical properties of the R cells.

Subthreshold test currents with sinusoidal or pseudorandom modulation were injected into one soma - the "driver" cell - and the electrotonic potentials were measured from both. The impedance Z(f) at the driver cell, and the coupling ratio H(f) (electrotonic voltage ratio of "follower" cell to driver cell) were measured as transfer functions on the frequency range f= 0.2 Hz to 128 Hz, and were compared with corresponding properties of the resistive and cable-coupled theoretical models.

At high frequencies, electrotonus of the follower cell consistently lagged the driver cell by more than 90°, so that the phase ∠H(f) of H(f) crossed -90° with significantly negative slope. This finding is consistent with the cable model but not with the resistive model, and we conclude therefore that a finite cable segment between the R cell somata better describes the coupling. Quantitative estimates of the electrical parameters were derived from 4 specific transfer function values (as mean ± SEM): D.C. resistance Z(0)=11.3±1 MΩ; D.C. coupling ratio H(0)=0.26±.01; slope d(∠H(f))/d(log f) with which phase ∠H(f) crosses -90°, -117±12°/decade; frequency f for ∠H(f)= -45°, 6.2±.2 Hz. These yield 4 model parameter estimates: cable segment length LN = 1.25 x length constant; hemi-infinite cable conductance = 0.75 x soma conductance; soma resistance Rl = 20 MΩ; soma capacitance C= 1.3 nF. For an assumed typical soma radius of 35 μm and cytoplasmic resistivity 79 Ω cm, these model parameters correspond to an equivalent coupling cable of radius 2.6 μm and length 810 μm, with specific membrane resistance R_m= 3.1 kΩ cm^2 and capacitance C_m= 8.6 ΩF/cm^2. (Supported in part by USPHS Grant HL 19995 and NIH Grant NS 11482 and by the Grass Foundation)

194.12 DENDRITES OF A BARNACLE NEURON ARE EXCITABLE: DETERMINATION WITH VOLTAGE-SENSITIVE DYES. V. Krauthamer* and W.N. Ross. Dept. of Physiology, New York Medical College, Valhalla, NY 10595

Optical recording techniques using voltage-sensitive dyes were used to simultaneously monitor potential changes at 32 different locations on individual neurons in intact ganglia. These recordings were correlated with specific positions on the cell by injecting the fluorescent dye Lucifer Yellow into the soma. Using this technique, in combination with standard intracellular and extracellular electrical measurements, we determined the locations of action potential initiation and the spread of passive potentials when current was injected into the cell body.

Supraesophageal ganglia from the giant barnacle Balanus nubilus were stained with the voltage-sensitive dye NK2367 and mounted on the stage of a compound microscope. The preparation was imaged with a x40 water-immersion lens onto a 10 x 10 square array of photodiodes, each element corresponding to 40 x 40 μm^2 in the object plan (Grinvald, Ross and Farber, P.N.A.S., U.S.A. 78: p. 3245-3249 (1981). Cells were impaled with a microelectrode and action potentials and hyperpolarizing potentials were evoked repetitively. The resulting changes in absorption, detected on each element at 750 ± 25 nm, were recorded on a laboratory computer. Signal averaging and timing established that the optical changes resulted from potential changes on only the stimulated neuron. Clear signals were detected from most positions on the cell, including fine processes.

When current is injected into the soma action potentials appear first in the dendritic tree and later in the soma. The earlier time-to-peak shows that the spike must be initiated in this region and hence this region contains active membranes. Action potentials with the same early time-to-peak as in the processes were also recorded from a point on the axon within the ganglion. Controls established that the recordings from the dendrites could not be due to signals coming from the axon. Therefore, we conclude that the action potential was simultaneously and separately initiated in these two regions of the cell. The implications of excitable dendrites for synaptic integration in these cells remains to be explored.

Supported in part by UPHS grant NS16295, Fellowship NS06929 (to V.K.) and the Irma T. Hirschl Foundation.

194.13 THE CRAYFISH MEDIAL GIANT NEURON: AN ELECTROTONIC MODEL AND SYNAPTIC PHYSIOLOGY OF IDENTIFIED DENDRITES. R.M. Glantz and T. Viancour Dept. Biology, Rice University, Houston, Tx. 77001

A single spike in the crayfish Medial Giant neuron, (MG) is sufficient to release a complete escape reflex. Since this response is elicited exclusively by cephalic sensory input, its occurrence is substantially dependent upon the integrative properties of the MG cephalic neurites. In order to gain further insight into the integrative mechanisms we examined the MG's dendritic geometry and passive cable properties. With 3 micropipettes inserted along the axon the MG input resistance (R_N= 35,800 ± 1600 ohms (±S.D.)) length constant (λ= 4.40 ± 0.95 mm), time constant (τ= 2.72 ± 1.35 ms) and axon diameter (p = 208 ± 34μm) were directly measured in 5 animals. The specific membrane resistance(Rm = 2016 ± 234 ohms), the axoplasmic resistivity (R_i= 59.0 ± 30.0 ohms) and the specific membrane capacitance (Cm + 1.42 ±0.93 μ f) were derived from these measurements.

Dendritic geometry was examined in 15 MGs filled with Lucifer Yellow-CH and photographed in a fluorescence microscope in serial focal planes in whole mount. The length and several diameter measurements were obtained for every neurite in each neuron. These dimensions were used to calculate an average geometric MG with neurite lengths, diameters. tapers etc. reflecting mean values of the sample.

By combining the biophysical meausrements with the averaged geometric dimensions we created a synthetic MG neuron by assuming that the passive properties of axonal and dendritic membrane were identical. The MG geometry was compartmentalized and the R_N at the base of the dendritic tree (integrative segment) and at varying distances from the dendritic tree was calculated by an iterative procedure (Rall, 1959, Exptl Neurol. 1:491-527) which yielded values in good agreement with our measurements. As a further test of the model, EPSPs and electrontonic potentials were simultaneously measured at the integrating segment and at various points along the axon cylinder. The measured and calculated attenuations were in good accord.

The MG electrotonic structure has several notable features: a) The most remote dendritic branches are only 0.75λ from the integrating segment; b) The summed dendritic input conductance amounts to only 10% of the input conductance of the integrating segment while the axon contributes 57%.

When excited by monosynaptic inputs from the cephalic roots, the integrating segment exhibits an 11-14% increase in input conductance. Previous studies have shown that monosynaptic inputs are restricted to single lobes of the brain and must therefore converge on identified branches of the MG dendritic arbor. Our calculations indicate that for a ΔG of even 10% to appear at the integrating segment the affected neurite must exhibit an 11.4 fold increase in G_N and a 130 fold increase in specific membrane conductance. Thus dendritic R_M decreases from 2016 to 16 ohm. cm^2. As expected from these considerations postsynaptic spatial summation is markedly nonlinear. Supported by N.S.F. Grant No. BNS 79-10335.

194.14 HIGH AFFINITY BINDING OF [^{3}H]PHENCYCLIDINE TO A MEMBRANE PREPARATION FROM INSECT MUSCLE. A. T. Eldefrawi, M. T. Filbin* and M. E. Eldefrawi*. Dept. of Pharmacology and Exp. Ther., University of Maryland School of Medicine, Baltimore, MD 21201.

Binding of [^{3}H]phencyclidine ([^{3}H]PCP) to a membrane preparation from housefly thorax was measured in 5 mM Tris-citrate, pH 7.1, using a filter assay. Specific [^{3}H]PCP binding was defined as that displaced by an excess (x 1000) of unlabeled PCP. Displaceable binding was abolished by placing the tissue in a boiling water bath for 2 min prior to incubation with [^{3}H]PCP. Specific [^{3}H]PCP binding was saturable both with time and at [^{3}H]PCP concentrations higher than 20 nM and increased linearly with tissue concentration. Binding was of high affinity. Scatchard analysis of data obtained from equilibrium binding studies revealed a single binding component with a K_d of 11.09 nM and B_{max} of 3 pmol/mg protein (28 pmol/g original tissue). The K_d obtained from the on (k = 1.22 x 10^5 s^{-1}) and the off (k_{-1} = 1.027 x 10^{-3} s^{-1} M^{-1}) rate constants was 8.4 nM. Neither carbamylcholine nor L-glutamate alone, or in the presence of mM concentrations of ATP, GTP, cAMP and cGMP, had any effect on [^{3}H]PCP binding. Specific [^{3}H]PCP binding was inhibited by the six PCP derivatives tried (IC_{50}'s nM-μM). Specific binding was also inhibited by calcium and by the following calcium antagonists: verapamil, nicardipine, nifedipine, +D600, -D600 and lanthanum, with IC_{50}'s in the μM-mM range. When the membrane preparation was stored overnight at 4°C, specific binding decreased by 15-20%, even when stored in the presence of one of the following antiproteases: EDTA, pepstatin, trypsin inhibitor or phenylmethane sulphonyl fluoride (PMSF). It is suggested that [^{3}H]PCP binds with high affinity and specificity to a calcium-binding protein. (Supported in part by NIH grant ES02594 and Army Research Office grant DAAG 29-81-K-0161.)

194.15 EFFECT OF LANTHANUM IONS ON NEUROMUSCULAR TRANSMISSION IN THE INSECT. H. Washio and T. Miyamoto.* Lab. of Neurophysiology. Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo, Japan 194.

The effect of extracellular lanthanum on neuromuscular transmission was studied in leg muscles of cockroach, Periplaneta americana, and segmental muscles of larval mealworm, Tenebrio molitor. Although lanthanum is more effective than magnesium is in suppressing evoked transmitter release, the frequency of miniature postsynaptic potentials (MEPSPs) was substantially increased in the presence of 0.1 mM La^{3+}. The potentiation of MEPSP frequency by La^{3+} was suppressed in a high Ca saline and enhanced in the absence of Ca^{2+}. An inhibitory action on the spontaneous release of transmitter which had been found in the presence of cobalt (Washio, H., J. Exp. Biol., in press) was not observed in a saline containing lanthanum. Lanthanum ions blocked insect neuromuscular transmission at a concentration as low as 0.05 mM. The quantum content estimated by the failure method was reduced by 80% in the presence of 0.1 mM La^{3+}. Thus, the reduction in the EPSP amplitude produced by La^{3+} is due to a decrease in the amount of transmitter release by a nerve impulse. The excitatory postsynaptic current (EPSC) was measured with a voltage clamp method during neuromuscular transmission and the falling phase was exponetial in a lanthanum-saline. The time constant of EPSC decay was increased appreciabley in the presence of 0.1 mM La^{3+}, despite a decrease in the quantum content. The response to L-glutamate applied iontophoretically was also reduced in the presence of lanthanum. External recording of MEPSPs has shown that adding lanthanum to the bathing medium increased the half-decay time of the potentials. These results suggest that lanthanum may affect the sensitivity of the glutamate receptor-ionophore complex in addition to its prejunctional action in insect muscle fibers.

195.1 A THERMORECEPTOR POTENTIAL IN PARAMECIUM TETRAURELIA. T.M. Hennessey*, Y. Saimi*, and C. Kung*. Lab. of Molec. Biol. and Dept. of Genetics, Univ. of Wisc., Madison, Wisc. 53706.

Wild-type Paramecium tetraurelia avoid temperatures from 37° to 42°C when tested in a buffered 1 mM calcium solution (Hennessey and Nelson, J. Gen. Microbiol. 112:337, 1979). A graded change in the membrane potential (ΔV_m) of up to 20 mV more depolarized than the resting level has been recorded intracellularly in the same solution by raising the temperature of a temperature-controlled bath. We refer to this ΔV_m in response to heat as the "thermoreceptor potential". The ΔV_m can be sustained for up to 1 min at constant high temperatures, but is readily reversible upon cooling. Spontaneous action potentials are also seen riding on the thermoreceptor potential at high temperatures.

The calcium-mediated action potential mechanism, localized in the cilia, is not involved in the generation of the thermoreceptor potential in P. tetraurelia. An identical $\Delta V_m/\Delta T$ of 1.3 mV/C° can be seen in wild type, deciliated wild type, and in a calcium channel mutant (pwB). A similar analysis in P. multinucleatum and P. caudatum has shown that the ciliary calcium channels are not involved in the depolarization in response to cooling (H. Toyotama, Ph.D. thesis, Osaka Univ., 1980). Thus, it appears that thermoreception, like mechanoreception (Ogura and Machemer, J. Comp. Physiol. 135:233, 1980), involves ion channels on the body membrane of Paramecium.

A behavioral mutant, teaB, shows a poor behavioral response to high temperatures as well as a significantly smaller $\Delta V_m/\Delta T$ of 0.6 mV/C°. Some of the behavioral and electrophysiological defects of teaB have been described previously (Satow and Kung, J. Membrane Biol. 59:179, 1981). An analysis of this mutant, as well as other possible thermosensory-minus mutants (E. Richard, unpublished) will aid in the analysis of the thermosensory transduction pathway in Paramecium.

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195.2 ECCENTRIC CELL DYNAMICS IN THE INTACT LIMULUS COMPOUND EYE, G.H.RENNINGER. Biophysics Interdept. Group, Dept. of Physics, Univ. of Guelph, Guelph, Ontario N1G 2W1, Canada.

Under uniform illumination sustained oscillations can develop in the optic-nerve response of the horseshoe crab's compound eye when it is left in situ (Barlow, R.B., Fraioli, A., J. Gen. Physiol., 71:699,1978; Renninger, G.H., Barlow, R.B., Soc. for Neurosci. Abstracts, 5:804(#2715),1979). These result from a synchronization of nerve-impulse generation between eccentric cells which communicate by means of lateral inhibition. Lateral inhibition is delayed with respect to the impulse initiating it (Ratliff, F., et al., Vision Res., 14:1155,1974) and is strong in the intact eye.

Eccentric cell membrane events underlying impulse synchronization in intact eyes were observed by means of glass intracellular electrodes (resistance ≅ 20 MΩ; cell resistances ≅ 10 MΩ). Each eye was uniformly illuminated and hyperpolarizing current (up to 2 na) was injected to reduce or eliminate impulse generation. At low light intensities the membrane potential showed random fluctuations similar to those reported in cells illuminated alone. At light intensities which can produce oscillatory optic-nerve responses, however, the membrane potential became more regular and an oscillatory component appeared. Frequencies of oscillation were 6-7 s^{-1}.

Simultaneous observations of eccentric cell membrane potentials and of the aggregate optic-nerve response were also made, allowing a direct confirmation of the correlation between synchronized activity among optic-nerve fibers and oscillatory membrane components and permitting the conclusion that these components are an oscillating hyperpolarization due to lateral inhibition arising from the optic-nerve discharge. These observations also provided a measurement of the phase relationship between the oscillations. At the higher intensities the optic-nerve response consists of bursts of activity separated by intervals containing few impulses. In such cases it was possible to measure the delay between the onset of lateral inhibition due to a burst and the occurrence of the first impulses in the burst. After correction for the impulse conduction delay in optic-nerve fibers between the intracellular and optic-nerve electrodes, which corresponded to a conduction velocity of ca. 2 ms^{-1}, this delay turned out to be 45±10 ms. The delay in inhibition permits synchronization of impulses; it equals about 30% of the period of the resulting oscillations. Previous theoretical calculations (Coleman, B.D., Renninger, G.H., J. Math. Biol., 3:103,1976) suggested that the delay in lateral inhibition should be significantly smaller than the values (≅ 100 ms) reported for excised eyes. (NSERC Canada Grant #A6983)

195.3 STRUCTURE OF LIMULUS RHABDOM IN LIGHT AND DARKNESS: RHABDOM TURNOVER IN THE NATURAL STATE. S. C. Chamberlain* and R. B. Barlow, Jr. (SPON: R. Verrillo). Institute for Sensory Research Syracuse University, Syracuse, NY 13210

We have previously reported (Science 206:361-363) that the rhabdom of the Limulus lateral eye is transiently broken down and rebuilt at first light onset each day. This process requires that efferent input from the central circadian clock precede first light onset. Since the original experiments were performed in the laboratory with sudden light onset, we have now examined the structure of the rhabdom of animals freshly caught and fixed in the natural environment. We find no essential differences between the ultrastructure of the rhabdom turnover process observed in the laboratory and rhabdom turnover as it occurs in nature.

In late June, transient turnover of the lateral eye rhabdom occurs at some point in time between 0400 and 0600. Synchrony from animal to animal is poor, but the synchrony among ommatidia within a given retina is quite good. At this time of day, the efferent fibers are still active, and the overall retinal structure is still that of nighttime. Apparently relatively little light is required to trigger rhabdom turnover, and processes other than sudden light onset must be responsible for synchronizing the process across the retina.

Quantification of the amount of microvillar membrane in the rhabdom at each point in time revealed that rhabdom breakdown represents a 3.5 times transient reduction in the area of photosensitive membrane. The maximum amounts of microvillar membrane are found early in the morning just before rhabdom turnover, and late in the afternoon (1800). During progressive light exposure through the course of the day, the area of membrane in the rhabdom increased in agreement with our earlier studies in the laboratory. We conclude that the rhabdom is maximally organized and contains the largest area of microvillar membrane when it is in darkness at night or when it is in light during the day.

In a parallel study we investigated the amount of efferent input necessary to prepare the rhabdom for turnover at first light onset. Animals were placed in darkness in early evening, then exposed to dim red light for 5 min before being fixed at 2 hr intervals. The results suggest that 3 to 5 hrs of efferent activity must precede light onset for rhabdom turnover to occur.

While both light and efferent input are required for rhabdom turnover, the amounts of each are relatively small, allowing the process to occur in nature as early as 0400 when the light intensity is low, and the daily period of efferent activity is not yet concluded.

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195.4 QUANTITATIVE ELECTRON PROBE MICROANALYSIS OF LEECH PHOTORECEPTORS. B. Walz* and A.P. Somlyo* (SPON: P.A. Liebman). Abt. f. Vergl. Neurobiologie, Univ. Ulm, D-7900, FRG and The Pennsylvania Muscle Institute, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

Leech photoreceptors exhibit a peculiar compartmentalization. Each cell contains a large "vacuole" into which the photoreceptive microvilli protrude. The "vacuole" is connected with the extracellular space over narrow (~20nm) clefts (septate junctions). The ionic composition of the cytoplasm and "vacuole" have been unknown. We used electron probe microanalysis of dry, ultrathin cryosections to determine quantitatively the elemental composition of the cytoplasm, "vacuole" and extracellular space (ECS). The data collected from five leeches are shown below:

		mmoles/kg dry wt. ± S.E.M.					
	n	K	Na	Cl	S	Mg	P	Ca
CYTO.	18	393±30.6	130±14.7	216±13.8	332±13.5	40±3.0	571±14.5	9±1.2
VAC.	17	50±9.8	552±80.4	216±57.3	304±15.3	1±3.5	77±7.6	28±2.1
ECS	15	74±6.2	997±77.3	826±68.0	275±18.2	14±3.4	28±5.0	33±2.9

The use of Thiery's silver cytochemical stain showed a positive reaction in the "vacuole," suggestive of carbohydrate rich material.

Our results show that: 1) the composition of the "vacuole" is similar to that of the extracellular space, as shown by the comparable Na/K (11 to 13) and K/Ca (1.8 to 2.2) ratios in these two compartments; 2) the "vacuoles" have a high S content and a relatively large deficit of Cl compared to $[Na^+]+[K^+]+2[Ca^{2+}]$; 3) the cytoplasmic K concentration is comparable to that in other nerve cells, and the Cl concentration is relatively high: significantly ($P<0.001$) higher than the cytoplasmic Na.

We conclude that the "vacuole" of the leech photoreceptor is in ionic communication with the extracellular space, as suggested by electrophysiological studies (Lazansky, A. & Fuortes, M.G.F. J. Cell Biol. 42: 241-252, 1969), and contains sulfonated glycosaminoglycan(s) that can partially exclude Cl; electroneutrality is maintained in part by organic anions, presumably sulfate. The high cytoplasmic Cl content is in excess of that predicted by passive distribution, and suggests the operation of a Cl pump.

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195.5 RESPONSE PROPERTIES AND OUTPUT EFFECTS OF IDENTIFIED MECHANOSENSORY INTERNEURONS IN THE CRAYFISH, PROCAMBARUS CLARKII. M.R. Plummer, J. Tautz, and J.J. Wine, Neurosci. Prog. and Dept. of Psych., Stanford Univ., Stanford, CA 94305.

The terminal abdominal ganglion of the crayfish contains approximately 50 bilateral pairs of projecting sensory interneurons which get input chiefly from the animal's tailfan. Of these, 22 have been uniquely identified on the basis of their structure, modality and physiological properties (Sigvardt, K.A., Hagiwara, G. & Wine, J.J., J. Comp. Physiol., in press). Interneurons with similar properties have been grouped together into six classes. We are analyzing the response properties and outputs of these interneurons, concentrating on two of these classes. One class (water movement-sensitive cells) responds best to directional water displacements, does not adapt, and responds poorly to touch of the animal's carapace. The other class of cells (touch cells) also responds to water movements but adapts, and responds strongly to touch. We have constructed a device with which we can present waterborne stimuli of known frequency and amplitude to the tailfan while recording intracellularly from the sensory interneurons.

With regard to the response properties of these cells, we have found that different interneurons have different frequency response characteristics and that certain neurons are excited at some frequencies and inhibited at others. In general, the touch cells have much higher thresholds than the water movement-sensitive cells although the touch cells will respond to higher frequencies than the water movement-sensitive cells.

With regard to output, we have found that some interneurons, when depolarized intracellularly, influence the activity of motor neurons in the rostral abdominal ganglia as well as in the terminal ganglion. To date, all of the neurons with these effects have been touch cells. For example, depolarization of one particular touch cell activates the peripheral inhibitor of the tonic flexor muscles (fI). When a 100 Hz water oscillation is used as a stimulus both the interneuron and fI are excited. Since the touch cell excites fI, it could be responsible for the response seen in the inhibitor. Hyperpolarization of the touch cell so that it does not fire during stimulus presentation results in a reduction of the response in fI. Therefore, one function of the touch cells may be to mediate postural changes in response to sensory stimuli.

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195.6 CENTRAL PROJECTIONS OF AFFERENT AXONS IN THE ANTENNAL NERVE OF LARVAL *MANDUCA SEXTA*. K. S. Kent* and J. G. Hildebrand, Dept. of Biological Sciences, Columbia University, New York, N. Y. 10027.

As background for studies of the postembryonic development of the antennal pathway in the central nervous system (CNS) of the moth *Manduca sexta*, we have traced the central projections of axons of the antennal nerve (AN) in fifth-instar larvae. We selectively stained one or more sensory neurons with cobalt sulfide followed by Timm's intensification of wholemounts. We observed that axons from neurons innervating tactile hairs on the larval antenna and on the head capsule near the antenna enter the brain via the AN and ramify as they run toward the circumesophageal connective (CEC). The axons then run through the CEC to the subesophageal ganglion, where they have additional characteristic arborizations. Cells innervating the other classes of antennal sensilla project to two other regions in the brain. One of these regions, medial to the point at which the AN enters the brain, is a nodular ball of neuropil that appears to be densely innervated by many fine fibers. Selective staining and ablation experiments suggest that this neuropil structure is the larval antennal center (LAC). A few "through fibers" extend beyond the LAC in the brain. These "through fibers" might be primary afferent axons or axons of higher-order cells in the antennal pathway that have been stained trans-synaptically. The other region of neuropil receiving fibers from non-tactile sensilla is ventral to the LAC, and its volume and density of innervation are much less than those of the LAC. We believe that these two regions of neuropil receive antennal chemosensory inputs.

When all classes of axons in the AN were stained in a preparation, additional central projections were apparent. Several fibers enter the brain and ramify diffusely close to the AN. Both these fibers and the "through fibers" described above are candidates to be the axons from the neurons in the antennal imaginal disk that are believed to provide contact guidance for the centripetal growth of adult antennal-sensory fibers during adult development (Sanes and Hildebrand, *Wilhelm Roux' Archiv 178*, 71, 1975).

These studies have been supported by NIH grant AI-17711 and NSF grant BNS 80-13511.

195.7 PHOTORECEPTOR SPECIFIC ANTIGENS: LOCALIZATION AND IDENTIFICATION BY MONOCLONAL ANTIBODIES. Norbert E. Kremer, Gail H. Stewart* and Fulton Wong*. Marine Biomedical Institute, UTMB, Galveston, TX 77550 and Dept. of Biochemistry, University of New Hampshire, Durham, NH 03824.

Monoclonal antibodies specific for photoreceptors of the squid have been isolated by screening hybridomas obtained from mice immunized with the whole squid retina. Potential antibody-producing clones were first screened by an ELISA assay and further selected by an indirect immunocytochemical method. These monoclonal antibodies stain the photoreceptors but not other cell types in the visual system of the squid. The photoreceptor-specific antigens localized by the monoclonal antibodies were identified as particular bands or dots on nitrocellulose transfers generated from one-dimensional or two-dimensional gel electrophoresis of squid retinal proteins. A set of twelve monoclonal antibodies studied and reported on here can be characterized broadly according to their immunocytochemical staining patterns: 1) those staining the outer segments of the photoreceptors; 2) those staining the whole cell, and 3) those staining preferentially the inner segments of the cells. Of the antibodies studied, in the same categories, there are some which give the same staining pattern but identify different polypeptides on the nitrocellulose transfers.

We sought to further identify the antigens localized by the various antibodies. Two antigens have been identified as different forms of rhodopsin. One form is localized by an antibody to the outer segment, while the other form is localized by other antibodies to the entire photoreceptor, including the axon and terminal region in the optic lobe. Analysis on SDS polyacrylamide gels shows that the first form runs as a diffuse band of approximately 47K molecular weight and has a Concanavalin A binding site. The second form migrates as a sharp band at 45K molecular weight and does not bind to Concanavalin A. Both forms become fluorescent upon reduction of extracts with $NaBH_4$, indicating presence of a retinyl chromophore. The localizations of these two forms of rhodopsin may have functional implications. The form which runs as a diffuse band (localized to the outer segment) must be involved in phototransduction. Further study of the protein which migrates as a sharp band (localized to the entire photoreceptor) may give clues to rhodopsin biosynthetic or degradation processes. (This research was supported by BNS 81-07067 and EY-03308 to F.W.)

195.8 THE NEUROBIOLOGY OF CALCIUM SENSITIVITY IN HERMIT CRABS. Karen A. Mesce, Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

Hermit crab shell selection involves a complex sequence of behaviors culminating in a decision about which shell to inhabit. The presence of calcium at the shell surface promotes the exploration of the shell leading to its selection (Mesce, Science 215:993, 1982). The detection of calcium at a shell surface by Pagarus hirsutiusculus hirsutiusculus requires physical contact. Such a requirement serves to increase the signal to noise ratio in a marine environment that already contains approximately 10 mM calcium.

To understand the neural basis of calcium reception in hermit crabs, both morphological and physiological aspects of possible chemosensory structures were examined. Using the staining technique of Slifer (Ent. News 71:179, 1960) to locate putative chemoreceptors, hairs on the legs, antennae, antennules and mouthparts were found to stain within seconds with an 0.5% solution of crystal violet. Subsequently these and other non-hairlike structures that stained quickly were examined at the electron microscopic level.

Cinematographic analysis shows that the antennules and thoracic appendages come into extensive contact with shell surfaces during shell exploration. Surgical ablation of antennules, antennae and mouthparts does not significantly alter shell selection behavior. However, if such ablations are paired with applications of distilled water to the leg hairs, shell selection is disrupted. Distilled water selectively damages chemoreceptive sensilla in lobsters (Derby and Atema, J. Exp. Biol., in press).

Multi-unit recordings from neurons that innervate appendage hairs and antennular aesthetascs verified that such structures are chemosensory. In the case of leg chemoreceptors, there was a high level of tonic background activity. When calcium solutions in the range of 10-40 mM were applied to leg sensilla, a variety of neural responses were obtained. In some preparations there was an increase in neural discharge in response to calcium solutions and in others there was a depression of activity to levels below pre-stimulus treatment. Similar results have also been obtained by Seelinger (J. Comp. Physiol. 113:95, 1977), who examined the responses of isopod chemoreceptors to calcium solutions.

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196.1 AXONS HOMOLOGOUS TO THOSE OF THE DROSOPHILA GIANT FIBER PATHWAY OCCUR IN MUSCA AND SARCOPHAGA. David G. King. Departments of Anatomy and Zoology, Southern Illinois University, Carbondale, Illinois 62901.

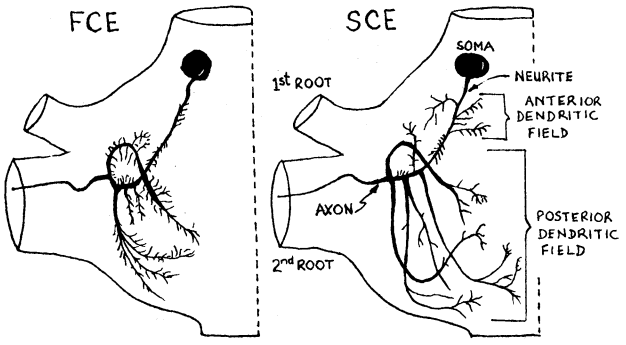
The dipteran giant fiber pathway, first described by Power (J. Comp. Neurol. 88:347), consists of three pairs of conspicuous axons. In Drosophila melanogaster a cervical giant fiber neuron (Koto et al., Br. Res. 221:213) or giant descending neuron (Strausfeld & Nassel, Handbook of Sens. Physiol. VII/6B) sends a large axon from the brain into the thoracic ganglion. Here the cervical giant fiber contacts two other axons. One of these, a thoracic interneuron, makes peripheral synapses onto the dorsal longitudinal muscle motor axons (King & Wyman, J. Neurocytol. 9: 753). The other, a motor axon, innervates the tergotrochanteral muscle.

The cervical giant fiber has homologues in several species of flies (Strausfeld & Nassel, op. cit.). Valentino (Neurosci. Abs. 3:190) has reported synapses similar to those of the peripherally synapsing interneuron in the posterior dorsal mesothoracic nerve of Musca domestica. In the present study microscopic examination of Musca and of Sarcophaga bullata has confirmed the presence of homologous synapses in both species. The axon which forms terminal axoaxonal synapses onto the dorsal longitudinal muscle motor axons follows a ganglionic course in Musca and in Sarcophaga which is indistinguishable from the course of the peripherally synapsing axon in Drosophila. Similarly, the ganglionic courses of the cervical giant fibers and the tergotrochanteral motor axons are similar in all three species. In Musca and Sarcophaga the cervical giant fiber may branch more extensively than in Drosophila (cf. Koto et al., op. cit., & Coggshall et al., Z. Naturforsch. 28:783). In Sarcophaga, the largest of the three flies studied, synapses homologous to those of the peripherally synapsing axon were located in the root of the posterior dorsal mesothoracic n., within the ganglion proper, rather than peripherally within the nerve. The central synaptic contacts among the three pairs of giant fiber pathway axons were not examined in this study.

The giant fiber pathway in Drosophila is a valuable system for genetic and developmental neurobiology, since several mutations are known to modify this pathway (Thomas, Neurosci. Abs. 6:742 & 7:543; Costello & Thomas, Neurosci. Abs. 7:543; Thomas, Costello & King, Neurosci. Abs. 8). Comparative study of homologous pathways in additional species will permit broader investigation into the ontogenetic and evolutionary determination of specific neuronal features. Flies which display significant modifications of the giant fiber pathway (such as Glossina; see King, Neurosci. Abs. 7:410) may be especially interesting.

196.2 FAST AND SLOW LOBSTER MOTONEURONS HAVE UNIQUE DENDRITIC MORPHOLOGIES. C. K. Govind and R. H. Hill.* Scarborough Coll. Univ. Toronto, West Hill, Ontario, M1C 1A4, Canada and Boston Univ. Marine Program, Marine Biol. Lab., Woods Hole, MA. 02543.

The closer muscle in the claws of lobsters, Homarus americanus, is innervated by a fast closer excitor (FCE), a slow closer excitor (SCE) and a closer inhibitor motoneuron. Since each of the FCE and SCE is identifiable in the 1st thoracic ganglion by the location of its soma (Govind, C. K. and Lang, F., 1981 J. Exp. Biol. 94:329-339) we compared their morphology by injecting their soma with cobalt or Lucifer Yellow (Figure). Both neurons are similar in general morphology being monopolar with a single neurite orginating from a soma located in the antero-ventral region of the ganglion. The neurite travels vertically to the dorsal surface and along this surface, where it gives off its primary dendrites, to the 2nd root which it enters as an axon. The FCE and SCE motoneurons however differ in their dendritic morphologies in several respects. First the SCE has an anterior dendritic field consisting of 3-4 primary dendrites which is completely lacking in the FCE. Second the posterior dendritic field usually has 5 primary dendrites in the SCE but only 3 in the FCE. Third these posterior primary dendrites of the SCE are longer and extend further across the ganglion than their conterparts in the FCE. Fourth while the axon of the SCE is a continuation of the neurite that of the FCE originates from one of the posterior primary dendrites. In summary the SCE has a more extensive and elaborate dendritic field than the FCE. This together with the peculiar origin of their axons may account for the lower firing threshold and prolonged motor patterns in the SCE than in the FCE of intact lobsters. Supported by NSERCC and MDA of Canada.



196.3 VARIABILITY IN THE OCULOMOTOR SYSTEM WITHIN AN ISOGENIC CLONE OF DAPHNIA MAGNA. T.R. Consi* and E.R. Macagno. Columbia University, New York, NY 10027.

Measurements of the number, position, structural features and synaptic connectivity of neurons within isogenic populations have revealed both great constancy and variability of these parameters. For example, our observations on a parthenogenetic clone of Daphnia magna showed constant numbers of neurons in the compound eye and optic lamina, but variable branching patterns and numbers of synaptic sites among identified neurons in the optic ganglion. We report here our recent observation that the number of fibers in particular muscles of the oculomotor system varies among random individuals of our Daphnia clone, as well as within individual parthenogenetic broods.

Three bilateral pairs of muscles move the compound eye of Daphnia. The three muscles on each side of the head share a common origin on the carapace and insert dorsally, laterally and ventrally on the eye. The muscle fibers of the lateral pair, although small (maximum diameter is 4-6 microns at the nucleus), can be readily visualized in whole mounts using Nomarski optics. Using this technique we measured the number of fibers per lateral eye muscle on both sides of 72 specimens from our clone. This number was found to range from two to four, with three being most common. Individual animals often had different numbers on the left and on the right. The data is presented below.

LATERAL EYE MUSCLE FIBER NUMBER		NUMBER OF ANIMALS	%
RIGHT	LEFT		
2	2	7	9.7
2	3	13	18.1
3	2	14	19.4
3	3	36	50.0
3	4	0	0.0
4	3	2	2.8
4	4	0	0.0
	Totals:	72	100

The distributions of fiber numbers among the progeny of individual Daphnia were found to be similar to that of the general population and independent of the numbers of fibers in the mother's lateral eye muscles.

Observations with the electron microscope of a serially-sectioned animal with three fibers per lateral eye muscle revealed that a single motor neuron innervates each muscle. The motor axon trifurcates as it approaches the muscle, the branches running along each muscle fiber and forming numerous synapses. The innervation of lateral eye muscles with 2 or 4 fibers is being examined.

196.4 Identification Of Target Tissues Of Aplysia Neuron R14 By Long-Duration Pressure Injection of Cobaltic Hexamine Chloride. A.R. Rittenhouse* and C.H. Price. Department of Biology, Boston University, Boston, MA 02215

The axonal tree of the identified Aplysia neuron R14 in the parietovisceral ganglion (PVG) was traced to peripheral locations by intracellular injection of the heavy metal complex, $[Co(NH_3)_6]Cl_3$. Standard electrophysiological techniques were used to monitor the cell while injecting 200 mM $[Co(NH_3)_6]Cl_3$ by pressure and/or current. We found that $[Co(NH_3)_6]Cl_3$ entered R14 more easily than other metals, did not clog the microelectrode as other dyes often do, and was transported at a rate comparable to that of Lucifer Yellow (~1.5 mm/hr). After constant injection and incubation periods up to 24 hr, the Co^{2+} was precipitated with 5% ammonium sulfide. The tissue was dehydrated and cleared, leaving a permanent, grey to black staining of the soma, ganglionic axons, and finer peripheral processes up to 15 mm distal.

The large diameter, single axon arising from the cell body immediately branches into 3 major axons. One axon projects rostrally into the vulvar nerve (VN), and occasionally into the parietovisceral connective, bifurcates within the VN, and both exit this nerve to innervate the dorsal artery. A second axon travels the length of the branchial nerve where it ramifies in the sheet of muscle (MS) surrounding the base of the heart. A third axon traverses the PVG to the left hemiganglion where it branches into 4 or 5 axons. The pathways of these latter axons vary and may enter the pericardial (PCN), genital (GN), or spermathecal (SPN) nerves. However, their target tissues are invariant and include the ganglionic artery, the digestive gland sheath (DGS), the pericardial wall (PCW), the base of the heart (via the auricular nerve), and the gastroesophageal aorta (GEA).

On many of these tissues numerous varcosities of various sizes can be seen and are presumed release sites. Positive correlations between intracellular soma spikes of R14 with extracellular recordings of its peripheral axons show that R14 sends processes onto the ganglionic artery, anterior aorta, dorsal artery, GEA, MS, into the right and left efferent vein nerves, AUN, and DGS. All these tissues are involved in the flow of hemolymph in Aplysia and appear to be target tissues for R14. Thus, in addition to the well-described motor innervation of Aplysia circulatory tissues, our data suggests that R14 plays a prominent role in the control of cardiac output, perhaps similar to this neuron's glycinergic anterior aorta contractions (Sawada et al., Brain Res., 207: 486-490, 1981). Furthermore, the presence of endings in vascular tissue reinforces the idea that R14 may also secrete a physiologically active peptide.

196.5 **MEMBRANE INFOLDING IN AXONS FROM THE BUCCAL GANGLION OF APLYSIA CALIFORNICA.** Katherine Graubard and Kelly Bennett*, Department of Zoology, University of Washington, Seattle WA 98195

Most molluscan axons are not smooth cylinders, but instead have a less regular shape with invaginations of surface membrane into the axoplasm. In order to estimate membrane channel density or to compute cable properties of such axons, the total amount of plasma membrane must be known. In the past, unit membrane capacity has been seriously overestimated when membrane folds were ignored.

Two Aplysia, weighing 210g and 70g, were dissected in low-calcium, high-magnesium saline. Nerves were pinned so that axon bundles appeared straight within the nerve sheath. The tissue was fixed in: 2.5% glutaraldehyde, 0.1M s-collidine pH 7.4, 0.43M NaCl, 0.02M $MgCl_2$ at 4° C. Secondary fixation was in 1% OsO_4 with the same salts and buffer. Tissue was dehydrated in graded alcohols and embedded in epon. Shrinkage of the axon bundles within the nerve sheath did not exceed 12% and was probably much less.

Measurements were made from electron micrographs of transverse sections cut from the middle portions of the radular nerve and buccal nerves 1, 2, and 3. The perimeter and cross-sectional area of each sampled axon was measured. The diameter, d, is that of the circle which encloses the same area as is contained in the measured axon cross-section. The infolding factor is the number by which πd must be multiplied in order to compute the measured perimeter of the axon (a cylinder without membrane folds has an infolding factor of one).

An infolding vs diameter plot shows a tendency toward more infolding for larger diameters. This agrees with a previous study of the right connective of the abdominal ganglion (Graubard, Brain Res. 88:325-332, 1975) in which a wider range of axon diameters, in larger animals, revealed a power function relationship between diameter and infolding. This study finds somewhat less infolding at each diameter, and still less for the 70g animal than for the 210g one. We are now examining individual axons to determine if the point scatter is due to variations in infolding along the length of an axon or to some property of individual axons.

The four nerves examined show differences between each other and when compared with the right connective of the abdominal ganglion. Buccal nerves 1 and 2 contain large numbers of very small axons which are packed together without individual glial coverings. The other axons in these nerves and in nerve 3 show varying amounts of glial wrap, some of which is much more extensive than any seen in either the radular nerve or the right connective. (Supported by NINCDS grant NS 15697-03).

196.6 ARE DIFFERENT PROTEINS SYNTHESIZED IN DISTINCT DOMAINS IN A NEURON? Bruce Tedeschi* and David L. Wilson. Dept. of Physiology and Biophysics, Univ. of Miami Sch. of Med., Miami, FL 33101.

The R2 neuron of the invertebrate Aplysia californica was used to test whether different messenger RNA species are translated in distinct, limited domains within the cell. After 15 min. of labeling of the R2 neuron with ^{35}S-methionine, the cell soma was frozen in an ethylene glycol-seawater solution, removed from the abdominal ganglion, and divided into two or three parts. In some cases the whole cell was used. Proteins from the cell parts were extracted and then separated by two-dimensional gel electrophoresis. The labeled (newly synthesized) polypeptides were detected with Kodak XAR x-ray film following PPO imbedding for fluorography. For quantitative analysis, selected gel regions, corresponding to spots on the film, were cut out and counted in scintillation fluid.

A map of spots consistently seen in the label pattern of proteins from whole cells was made. Twenty six of these spots were examined on each of the gels from parts of cells. The analyzed gels were obtained from five R2 neurons, two of which had been divided into two parts after labeling, and three of which had been divided into three parts. Models were used to predict the ratio of counts in the various cell parts. There was no evidence for limited domains of synthesis within the cell for any of the 26 examined polypeptides.

Reservations concerning the generalizability of our result include: a) we have examined only one type of neuron, b) only the more abundantly synthesized polypeptides were examined, c) an even shorter labeling time might be necessary to avoid possible transport of the newly synthesized proteins from sites of synthesis, d) a wrap of glial cells is present surrounding the R2 neuron, and e) only the cell soma was divided into parts (It would be of great interest to compare the synthesis of proteins in the soma and dendrites of a vertebrate neuron, should an adequate system be found).

We conclude that a number of the more abundantly synthesized proteins in the R2 neuron do not appear to be synthesized in small domains within the protein-synthesizing regions of the cell. This result demonstrates the sensitivity of present techniques of protein analysis and presents restrictions on theories of how cytoplasm is organized.

196.7 INTRACELLULAR RECORDING OF BURSTING PACEMAKER ACTIVITY FROM INTACT APLYSIA. Gayne M. Bablanian* and Steven N. Treistman (SPON: S. Fredman). Univ. of Rhode Island, Kingston, RI 02881 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Neuron R15, found in the abdominal ganglion of Aplysia californica, has been extensively studied as a model for the generation and modulation of bursting pacemaker activity. However, the functional significance of this cell's activity pattern has not been established. In order to determine the functional significance of R15's bursting pacemaker pattern, we have been making intracellular recordings from the cell in intact Aplysia.

In these studies, the abdominal ganglion is exposed by a small, dorsal incision in an animal pinned foot-down in a paraffin-lined dish. The sides of the incision are pulled up and away, so as to allow access to the ganglion while preventing loss of hemolymph or mixing of hemolymph with the seawater surrounding the animal. Cell R15 and/or other cells may then be impaled with an intracellular micropipette electrode.

Using this preparation, we have found that in approximately 70% of impaled R15's, easily recognizable bursting pacemaker activity is present. The most consistent source of irregularity in burst activity is due to excitatory inputs which reach the abdominal ganglion via the right connective nerve. Cutting this nerve results in a greater regularity of bursting activity.

Some of the existing data supports the view that R15 is an integral part of an osmoregulatory response in Aplysia. We have examined this possibility in the intact preparation. Substitution of 90% sea water for normal sea water produces a hyperpolarization of R15 which either silences it or significantly increases the interburst interval over that produced by control bath changes in 75% of trials. In a number of trials in which slowing of the cell did not occur, increased EPSP activity was evident during stimulus presentation. In many of these cases, slowdown of R15 after hypotonic substitution was evident after cutting the right and left connective nerves to the head ganglion. Hypotonic sea water had no effect on cell R2. Our results show that bursting pacemaker activity is not an artifact of ganglion isolation and that a role for R15 in osmoregulation is possible. Supported by grant BNS 81-09348.

196.8 APLYSIA ATRIAL GLAND PEPTIDE(S): I. ACTIVATION OF NEURONAL INPUT INTO BUCCAL MUSCLE I5. II. STIMULATION OF EGG LAYING IN STYLOCHEILUS. Vanita A. Padgaonkar* & Jeffrey L. Ram (SPON: H. Goldman). Dept. of Physiology, Wayne State Univ., Detroit, MI 48201.

The atrial gland (AG) of Aplysia is a glandular structure at the rostral end of the large hermaphroditic duct. Like the egg-laying hormone (ELH) of the bag cell neurons, AG peptides cause egg laying upon injection into mature Aplysia (Arch et al., J. Comp. Physiol. 128:67).

I. We examined whether AG factors have effects similar to ELH on excitatory input into buccal muscle I5. In buccal ganglia-I5 preparations ELH has been shown to increase excitatory input into muscle I5 (Ram, Brain Res. 236:505). I5 electrophysiological input was recorded with an extracellular electrode in buccal ganglia-I5 preparations. The preparation was constantly superfused with buffered sea water (Instant Ocean containing 10 mM glucose, 5 mM tris (pH 8.0), and 100 µg/ml streptomycin) except that the pump was stopped for 10 min when samples were applied. Stopping the pump and adding comparable volumes of buffered sea water had no effect on I5 activity.

As little as 1/10000 of the AG of a single animal increased excitatory input to muscle I5. With higher concentrations, activity began within 10 min of sample application and typically peaked shortly after the pump was turned back on. The increased input to I5 lasted from half an hour to several hours, gradually declining to previous levels, and this excitatory response could be obtained again on subsequent applications. The factor(s) causing the response is/are stable to boiling. Comparable extracts made from gonad, small hermaphroditic duct, winding gland, esophagus, crop, posterior gizzard, liver, or rectum did not have excitatory effects similar to the effects of AG extracts on I5 input. CCK1-8 and LHRF also had no effect on I5 input.

AG was fractionated on Sephadex G-50 in formic acid, and fractions were subsequently freeze-dried. AG fractions most active in exciting I5 input eluted with approximately the same V_e/V_0 as ELH.

II. AG homogenates and Sephadex G-50 fractions were bioassayed for stimulation of egg laying by injection into Stylocheilus, which has been shown to lay in response to ELH (Ram, ibid.). Stylocheilus laid in response to 1/500 and 1/1000 AG but not following 1/2000, 1/5000, or 1/10000 AG. Blind injections of the same Sephadex G-50 fractions tested above for neurostimulatory activity gave egg laying only for neuroactive fractions.

Supported by NIH grant NS15041 to JLR.

196.9 A SECOND NEUROACTIVE SUBSTANCE FROM R3-R14 NEURONS: CHARACTERIZATION AND EFFECTS ON ANTERIOR AORTA OF APLYSIA. D. Gibson*, C.Y. Lin*, and D.J. McAdoo (spon.: R. Bowker). Marine Biomedical Institute, Univ. Tx. Med. Br., Galveston, Texas 77550-2772.

We believe that cells R3-R14 in the abdominal ganglion of Aplysia release at least two different messengers: glycine (Sawada et al. 1981, Brain Res. 207; Price et al. 1979, J. Neurobiol. 10) and peptides (Gainer and Wollberg, 1974, J. Neurobiol. 5). We are investigating the possibility that the peptides made in these cells have hormonal effects. We have extracted an acidic fraction of about 500 Dalton molecular weight from these cells. This fraction is active on aorta muscle, the apparent target of glycine, where it blocks excitatory junction potentials (EJPs) but does not affect inhibitory potentials (IJPs). Simultaneous recording from nerve and muscle and from multiple sites on the muscle indicate that the effect is post-synaptic, and it can be used to determine the focus of innervation of electrically-coupled muscle fibers.

Possible release sites of this material are processes of R14, which terminate on the aorta, and axons of R3-R14 which terminate at the gill efferent vein, on a direct path to the aorta via the heart. Our material is equally effective when applied to the outside of the aorta or to its luminal surface, whether extracted from cell bodies or from axons.

Separation techniques to date include sizing on a Sephadex G10 column, cation exchange on a Dowex column, and further separation with a reverse phase HPLC column, after which the recovered material remains active in doses down to 1/10 of the material from one animal.

Most recording was done with surface electrodes which could not be dislodged by muscle contraction. Intracellular microelectrodes were used to corroborate these records and to assay the effect of this material on resting potential.

In summary, it appears extremely likely that R3-R14 releases more than one chemical substance to act on circulatory musculature.

Financial support provided by DHEW Grant NS 1311, NSF Grant PCM 7-9-12175 and NRSA Training Grant 1-T32 NS 071185-01.

196.10 PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST BIOLOGICALLY ACTIVE PEPTIDES FROM THE ATRIAL GLAND OF APLYSIA CALIFORNICA. S.D. Painter, G.T. Nagle, K.L. Kelner and J.E. Blankenship. Marine Biomedical Inst., Univ. Tx. Med. Br., Galveston, TX 77550.

The atrial gland of Aplysia californica contains at least three peptides (A, B and ERH) which induce bag cell discharge in vitro and egg-laying behavior in vivo. It has not yet been shown, however, that endogenous A, B or ERH normally mediate these responses in the animal. Moreover, since the atrial gland appears to be an exocrine gland, releasing its contents into the lumen of the reproductive tract, we were interested in further studying the possibility of atrial gland secretion into the hemocoel and in locating alternative endogenous sources of these peptides.

A crude preparation of small molecular weight molecules was isolated from atrial gland homogenates by Sephadex G-50 column chromatography. Biologically active material (approximately 3000-15000 daltons molecular weight) was dialyzed against distilled water and then injected intraperitoneally into mice (0.5 mg in Freund's adjuvant/mouse/injection). Serum was collected and shown to contain antibodies against the natural biological material and against synthetic A, B and ERH (synthesized by Dr. D.H. Schlesinger of New York University), as detected by enzyme-linked immunosorbent assay (ELISA). The serum antibodies have also been used (1:1000 dilution) to specifically label the atrial gland immunocytochemically, using the unlabelled antibody peroxidase-anti-peroxidase (PAP) method.

To generate monoclonal antibodies, spleen cells were collected from immunized mice and fused with P3 myeloma cells by a modified method of Köhler and Milstein. Hybridoma cells were selected by growth in HAT medium. Supernatants from the resulting clones were screened for the presence of antibodies directed against the purified biological material and against synthetic A, B and ERH. The most interesting clones were selected and subcloned by limiting dilution. We now have several cultures which produce antibodies selective for B and ERH. These monoclonal antibodies will be used to investigate the source, release and function of these biologically active peptides by immunocytochemistry and radioimmunoassay. (Supported by NSF PCM 79-12175, NIH NS 11255, NS 07010, NS 07025, and NS 07185)

196.11 BIOSYNTHESIS OF LOW MOLECULAR WEIGHT PEPTIDES IN THE ATRIAL GLAND OF APLYSIA CALIFORNICA. K.L. Kelner and J.E. Blankenship. Marine Biomedical Inst., Univ. Tx. Med. Br., Galveston, TX 77550.

To date, three low molecular weight (MW) peptides, designated A, B and ERH, have been isolated from the atrial gland of Aplysia californica. Each peptide has been sequenced, is 34 amino acids in length, and all have isoelectric points between 8 and 9. All three peptides cause egg laying in the intact animal and, when applied to the isolated nervous system, induce a discharge of the neurosecretory bag cells. We have studied the in vitro biosynthesis of small peptides in the atrial gland by 3H-amino acid incorporation. Freshly dissected glands were incubated for 2 h at 15C in artificial sea water containing 55 mM glucose and 50 mCi of an 3H-amino acid mixture. Glands were homogenized in an acid medium containing peptidase inhibitors and centrifuged. Approximately 1 mg of the lyophilized supernate was applied to an SDS polyacrylamide gradient gel (8-15%). Scintillation counting of 2 mm gel slices revealed a predominant peak with a MW less than 5000 and multiple unresolved peaks at higher MW. The small MW peak contained about 35% of the label which had been incorporated into macromolecules. Alternatively, when 150 mg of the lyophilized supernate was applied to a Sephadex G-50 column, these small labelled peptides were resolved into two equal peaks of radioactivity with apparent MW of approximately 8000 and 5000. The 8000 MW peak was coincident with a small peak of absorbance at 280 nm (OD=0.5), while the 5000 MW peak co-eluted with a larger peak of absorbance (OD=1.3). When the material in these peaks was subjected to isoelectric focusing, the radioactivity from each peak was found as a single band with isoelectric points of about 9 for the 8000 MW peak and 4 for the 5000 MW peak. When quantitatively bioassayed for egg laying activity, the column effluent showed a single symmetrical peak of activity centered over the 8000 MW peak. Injection of the 8000 MW peak material into an animal from which the bag cells had been removed caused egg laying at approximately the same dose as in an intact animal. Since only ERH, but not peptides A or B is able to elicit egg laying in bag cell-less animals, this suggests that a large proportion of the 8000 MW material is ERH, not A and B. Further purification of the 8000 MW peak by DEAE ion exchange chromatography yielded a biologically active fraction which migrated as a single band on SDS gradient gels and which comigrated with synthetic ERH (synthesized by Dr. D. Schlesinger, New York University). In conclusion, we have demonstrated that the atrial gland of Aplysia californica is able to synthesize two classes of small MW peptides: 1) a basic peptide group which causes egg laying and which appears to be largely ERH, and 2) an acidic peptide group which has, as yet, no known biological activity. Supported by NSF PCM 79-12175, NIH NS 11255 and NS 07185.

196.12 FACTORS AFFECTING THE MORPHOLOGY OF IDENTIFIED MOLLUSCAN NEURONS GROWN IN CELL CULTURE. S.B. Kater, H.R. Miller* and C.S. Cohan*, Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Identified neurons from the buccal ganglion of the snail, Helisoma, express a distinctive morphology, physiology and connectivity within the surroundings of their normal ganglionic environment. We now report that these identified neurons can be removed from their complex environment and transplanted into the defined conditions of cell culture. We have begun to address the following questions: 1) do isolated cells retain features which enable them to be distinguished from one another, and 2) what factors affect the expression of cell-specific characteristics in culture. In order to answer these questions we have cultured neurons in either defined medium, medium which contained Helisoma conditioning factor (CF), or dishes in which CF was preadsorbed to the polylysine surface (Wong, et al, J. Neurosci. 1:1008, 1981).

In cell culture, neurons 4, 5, and 19 retained the characteristic action potential waveforms which distinguish them from one another in the ganglion. These waveform properties remained relatively constant throughout 7 days of culture and were independent of the extent of growth exhibited by each cell.

The morphology of cultured neurons was, as expected, quite different from that in the CNS. When co-cultured with Helisoma brains, isolated neurons 4 and 5 characteristically were multipolar and displayed diffuse neuritic sprouting which was radially arranged about the soma. Neuron 19, under the same conditions, displayed a very different morphology. There was much less outgrowth, it was usually restricted to one or two quadrants of the cell body, and associated with extensive preliminary veiling. Growth of 19 often stopped by day 1 or 2, whereas the growth of 4 and 5 may peak on days 4 to 6. When these same neurons were grown on CF adsorbed plates very different results were observed. Under these conditions neuron 19 displayed the profuse neuritic sprouting that was characteristic of 4 and 5 under co-culture conditions. Thus, neuron 19 has different requirements from neurons 4 and 5 for its growth.

Taken together these initial observations suggest that 1) the characteristic action potential waveforms of a neuron are independent of conditions which affect cellular morphology, and 2) different identified neurons respond differently to particular environmental cues which may evoke, retard, or modify the expression of neuronal morphology.

Supported by NS 15350 and grants from the Whitehall Foundation and MDA.

197.1 SYNAPTIC MONOCLONAL ANTIBODIES. 1. AN ANTIGEN WHICH HAS A HORMONE RECEPTOR-LIKE DISTRIBUTION IN THE RAT BRAIN. R.S. Lasher, L.N. Minier, P.F. Erickson, E.E. Mena and C.W. Cotman. Dept. Anat., Univ. Colo. Med. Sch., Denver, CO 80262; Dept. Psychobiol., Univ. Calif., Irvine, CA 92717

In an experiment designed to raise monoclonal antibodies (MABs) to synaptic antigenic determinants, a (Balb/C x C57/B1) mouse was immunized first with concanavalin A-binding proteins purified from synaptic junctions (SJ-CONA), and then with synaptosomal plasma membranes (SPMs) just prior to the fusion. Using a solid phase assay employing I-125 goat anti-mouse IgG+IgM, over 30 culture media were found which contained antibodies reacting with native SPMs, denatured SPMs and denatured synaptic junctions. Antibody from one culture produced a faint, patchy fluorescence over neuronal processes when living cultures of rat cerebellum were incubated first with medium and then with FITC-goat anti-mouse IgG+IgM. MAB from clone 2,9D of this culture was found to be an IgM by Ouchterlony immunodiffusion. It continues to produce the same fluorescent pattern with living cells as seen before cloning.

After indirect immunoperoxidase methods, examination with the light microscope revealed intense nuclear staining in pyramidal and granule cells in the hippocampus, Purkinje cells and deep nuclear neurons in the cerebellum, and in neurons in the caudate-putamen. Somewhat less intense nuclear staining was seen in neurons in most layers of the cerebral cortex, in the amygdala, medial thalamus, lateral mammillary area, and cerebellum (mainly basket-stellate cells and some granule cells). Correspondingly intense cytoplasmic staining was seen in these same areas in neurons lacking nuclear staining. After electron microscopy of the cerebellum, cerebral cortex and hippocampus, reaction product was seen to be uniformly distributed over the plasma membranes of many neuronal processes and synapses; it was also associated with smooth ER and mitochrondria in the cytoplasm, and with euchromatin in the nucleus. A small amount of product was seen in the cytoplasm of neurons whose nucleus contained large amounts of product. A few glial processes were also lightly stained.

MAB 2,9D also reacts with SJ-CONA, as well as with crude homogenates of rat liver, kidney, thymus, testes and skeletal muscle. The antigenic determinant is distributed in a pattern very similar to that seen for the binding of various hormones (e.g., corticosterone) in the rat brain, raising the possibility that it may be associated with a hormone receptor or a functionally similar type of molecule. Supported by NIH grants #NS-13133, NS-09199 and NS-08957.

197.2 ASTROCYTE SUBCLASSES DETECTED BY MONOCLONAL ANTIBODIES. C. Lagenaur. Inst. of Neurobiology, Heidelberg Univ., 6900 Heidelberg, F.R.G.

Three monoclonal antibodies have been identified that appear to be useful in defining subpopulations of central nervous system astroglia. The best characterized antibody, anti-SSEA-1, has been previously characterized as to its reactivity with the cell surface of mouse embryonic cells of morula and later stages (Fox, N. et al., Develop. Biol. 83:391, 1980). Two additional monoclonal antibodies, designated anti-M3 and anti-M4, were derived from rats immunized with mouse cerebellar membranes that had been fractionated via sucrose-deoxycholate gradient centrifugation. Using anti-glial fibrillary acidic protein as a marker for mature astrocytes, the cellular distribution of the antigens could be determined by indirect immunofluorescence. M3 was found to be expressed by nearly all astrocytes in primary cultures of early postnatal mouse cerebella; SSEA-1 was expressed by only 20-40% of all astrocytes. M4 was expressed by less than 10% of all astrocytes, becoming increasingly detectable after 3-5 days in vitro. Both SSEA-1 and M4 were not detectable on fibronectin positive fibroblast-like cells, while M3 was expressed by at least some of these cells. The comparison of in vivo and in vitro expression of these three cell surface antigens may prove useful in distinguishing relationships between astroglial subclasses.

197.3 MONOCLONAL ANTIBODIES RECOGNIZE SPECIFIC CELL TYPES IN THE DEVELOPING RAT CEREBELLUM. Joel M. Levine*, Phyllis Kawanabe* and William B. Stallcup*. The Salk Institute, Molecular Neurobiology Lab, San Diego, CA. 92138. (SPON: J. Patrick).

We have produced two monoclonal antibodies that bind to cell surface components of cells in rat cerebellar cortex at 2 different stages of development. We immunized mice with B49 cells, a clonal line with properties of both neurons and glia, and fused the spleen cells with the P3x63Ag8.653 myeloma line. Hybridomas were screened by indirect radioimmune binding to living B49 cells and by immunofluorescent staining of cerebellar cultures. Positive cells were cloned by limiting dilution.

Antibody D1.1 immunofluorescently labels cells of the external granular layer (EGL) as well as neuroepithelial cells throughout the brain. The D1.1 antigen is expressed by dividing cells (as shown by ^{3}H-thymidine autoradiography) as early as embryonic day 19 when the EGL begins to form. It is present throughout the period of cellular proliferation but is no longer detected after postnatal day 20 when the EGL disappears. No cells in the adult cerebellum are labeled with D1.1. Postnatal cerebellar cells in culture also express the D1.1 antigen. However, with increasing maturation in vitro, both neurons and astrocytes lose the D1.1 antigen. Crude preparations of gangliosides from B49 cells inhibit antibody binding suggesting that the D1.1 antigen is carried on a ganglioside. Similar preparations from cell lines that do not bind the antibody have no inhibitory activity.

Antibody D120.43 is directed against the NG2 surface antigen, a marker previously shown to be on the surfaces of subpopulations of the neurons and astrocytes surviving in cultures of postnatal cerebellum (Dev. Biol., 83:154). D120.43 binds to process bearing cells within the molecular layer of adult rat cerebellum as well as cells throughout the brain. On the basis of cell morphology and position, some of the D120.43 positive cells are identified as basket and stellate interneurons. In contrast to D1.1, D120.43 does not label proliferating cells of the EGL but rather it appears on cells as they begin to differentiate within the developing molecular layer. D120.43 immunoprecipitates 2 polypeptides of molecular weights 300,000 and 500,000 from detergent extracts of ^{125}I-labeled B49 cells.

The antibodies described here identify cell surface antigens that mark 2 different stages in the development of cerebellar interneurons: 1) a proliferative stage during which multipotent stem cells carry the D1.1 antigen on their surfaces and 2) a differentiated stage during which stellate and basket cells express the NG2 antigen. These latter 2 cell types retain the NG2 marker in the adult.

197.4 ATP-DEPENDENT Ca^{2+} TRANSPORT ACTIVITIES IN SYNAPTOSOMES: PURIFICATION OF A CALMODULIN-SENSITIVE Ca^{2+} PUMP FROM SYNAPTIC PLASMA MEMBRANES. Diane M. Papazian*, Hannah Rahamimoff*, and Stanley M. Goldin. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

Membrane fractions derived from osmotically lysed synaptosomes contain two nonmitochondrial ATP-dependent Ca^{2+} transport activities differing in their regulation by calmodulin and in their stability. Synaptic plasma membranes contain a labile, calmodulin-activated, (Ca^{2+} + Mg^{2+}) ATPase catalyzing ATP-dependent Ca^{2+} transport after reconstitution into liposomes. Synaptosomal vesicles, a fraction including but not exclusively composed of synaptic vesicles, contain a more stable, calmodulin-insensitive ATPase catalyzing Ca^{2+} uptake into native and reconstituted vesicles (Papazian, D.M. et al., Soc. Neurosci. Abstr. 7:367,1981).

The calmodulin-activated (Ca^{2+} + Mg^{2+}) ATPase of synaptic plasma membranes has been purified at least 50-fold by calmodulin-Sepharose-4B affinity chromatography. The purification procedure removes most of the Mg^{2+} ATPase activity. The major protein in the purified preparation, M_r = 140,000, has been identified as a (Ca^{2+} + Mg^{2+}) ATPase; it is phosphorylated by [γ^{32}P] ATP in a Ca^{2+} dependent manner to form an acyl (hydroxylamine-sensitive) phosphoenzyme. After reconstitution into liposomes, the purified enzyme efficiently catalyzes ATP-dependent Ca^{2+} transport, with a ratio of 1 Ca^{2+} transported per ATP hydrolyzed.

The synaptic plasma membrane (Ca^{2+} + Mg^{2+}) ATPase has been compared with proteins of M_r = 94,000 (major component) and 140,000 purified from synaptosomal vesicles by "transport specific fractionation" on the basis of their involvement in ATP-dependent Ca^{2+} transport (Papazian, D. et al., Proc. Natl. Acad. Sci. USA 76:3708, 1979). The M_r = 94,000 protein is hypothesized to comprise a distinct Ca^{2+} transport component, corresponding to the stable, calmodulin-insensitive Ca^{2+} transport activity of synaptosomal vesicles.

Thus, the M_r = 94,000 and 140,000 proteins may represent two distinct synaptosomal ATP-dependent Ca^{2+} transport systems, differing in their regulation by calmodulin, their subcellular localization, and their physiological roles within nerve terminals Supported by NIH Grant 16475.

197.5 COMPARISON OF THE HIGH AFFINITY CA^{2+}- STIMULATED MG^{2+}- DEPENDENT ATPASES OF RAT BRAIN SYNAPTIC AND MICROSOMAL MEMBRANES. M.L. Michaelis, E.K. Michaelis, H.H. Chang*, and T.E. Kitos*. Center for Biomedical Research, Univ. of Kansas, Lawrence, KS. 66045

The regulation of intraneuronal free calcium concentrations in the range of 0.1 - 1.0μM has recently been the subject of intensive investigation. Two ATP-utilizing systems may play a role in controlling intrasynaptosomal free Ca^{2+}: 1) Ca^{2+} sequestration by endoplasmic reticulum-like structures, and 2) Ca^{2+} extrusion from the nerve terminals via a plasma membrane Ca^{2+} pump similar to that in erythrocytes. Although high affinity (Ca^{2+} - Mg^{2+}) ATPase activity has now been reported to exist in both microsomal and synaptic plasma membrane fractions from brain, it has not yet been determined whether the synaptic plasma membrane high affinity Ca^{2+} - stimulated Mg^{2+} ATPase differs from that of the brain microsomal fraction. This report describes the use of highly purified rat brain synaptic plasma membranes to obtain detailed information about the properties of the synaptic membrane (Ca^{2+} - Mg^{2+}) ATPase in order to determine whether this enzyme has any characteristics which distinguish it from the microsomal enzyme.

The high affinity synaptic membrane (Ca^{2+} - Mg^{2+}) ATPase activity was found to be strictly dependent on the presence of Mg^{2+}, to have a high affinity for Ca^{2+} ($K_{0.5}$ = 0.23μM), to have a relatively high affinity for both Mg^{2+} and ATP ($K_{0.5}$ = 6.6μM for Mg^{2+} and K_M = 18.9μM for ATP), and to exhibit positive cooperativity in the activation of the enzyme by ATP (Hill coefficient of 1.5). The membrane (Ca^{2+} - Mg^{2+}) ATPase activity was not inhibited by ouabain or oligomycin, nor was it significantly affected by changes in K^+ or Na^+ concentrations in the incubation. The plasma membrane enzyme was, however, quite sensitive to low concentrations of vanadate (5μM). Examination of microsomal (Ca^{2+} - Mg^{2+}) ATPase under conditions identical to those used with plasma membranes, i.e., the presence of 5μM Mg^{2+}, 1 mM ATP, and a divalent cation chelating CDTA buffer system, revealed (Ca^{2+} - Mg^{2+}) ATPase activity with high affinity for Ca^{2+} in microsomes also ($K_{0.5}$ = 0.48μM Ca^{2+}). The basal Mg^{2+} ATPase in microsomes was 3-fold higher than in synaptic membranes, and thus the Ca^{2+} - stimulated activity represented a much smaller fraction of the total divalent cation ATPase activity in microsomes than in synaptic membranes. Unlike the plasma membrane enzyme, the microsomal Ca^{2+} - stimulated Mg^{2+} ATPase was not affected by 5μ vanadate. Thus the two enzyme systems do indeed have similar characteristics, but they appear to differ in their sensitivity to low vanadate concentrations and to exhibit slightly different affinities for Ca^{2+} under the assay conditions used in these studies. (Supported by grants NS 16364, AA 04732, and Ctr. Biomed. Res., Univ. of KS.)

197.6 PURIFICATION OF A MEMBRANE PROTEIN DISTRIBUTED IN A GRADIENT IN CHICK RETINA. Joseph R. Moskal*, G. David Trisler*, and Marshall W. Nirenberg. Lab. Biochem. Genetics, NHLBI, NIH, Bethesda, MD. 20205.

A 35-fold dorsal-ventral gradient of cell surface molecules (TOP) in chick neural retina, that can be used as a marker of cell position, has been identified by the use of a monoclonal antibody obtained by the fusion of P3X63 Ag8 myeloma cells with spleen cells from mice immunized with 14-day chick embryo dorsal retina (Trisler et al., Proc. Natl. Acad. Sci., (1981) 78, 2145). Antigenicity was destroyed by trypsin; however, trypsinized retina cells that were cultured for 24 hr bound 50% as much antibody as intact retina. TOP was purified as follows: dorsal retina cells were homogenized in hypotonic buffer and membranes were pelleted and washed by repetitive centrifugation at 30,000 x g. Particulate protein was solubilized (1% Triton X-100, 0.2% SDS, 1 mM phenylmethylsulfonylfluoride, 5 mM EGTA, 150 mM NaCl and 25 mM HEPES; pH 7.5) and the 30,000 x g supernatant fraction was applied to antibody-Affigel 10 columns that were prepared with antibody to TOP or antibody synthesized by parental myeloma, P3X63 Ag8, that had been purified by Protein A-Sepharose column chromatography. The antibody-Affigel 10 columns were washed with the above buffer followed by the same buffer with 0.1% rather than 1% Triton X-100, and bound material was eluted with a solution containing 0.2 M acetic acid containing 0.1% Triton X-100 and 150 mM NaCl; pH 3.0-3.5. Samples were prepared for SDS-PAGE according to Neville (J. Biol. Chem., (1971) 246, 6328) diluted 10-fold with Laemmli's electrode buffer (Nature, 227, 680), subjected to electrophoresis, and stained with silver (Merril et al., Electrophoresis, (1982) 3, 17). Only 1 major band with an M_r of 46,000-49,000 was detected in the eluate from the anti-TOP IgG-Affigel column; whereas, this band was not detected in the eluate from the P3X63 Ag8 IgG-Affigel column. Isoelectric focusing (pH 2.5-5.0) of affinity-purified TOP resulted in a sharply focused band at pH 4.0-4.1. Affinity-purified TOP from embryonic chick cerebrum and thalamus had the same molecular weight (SDS-PAGE) and isoelectric point as TOP purified from chick retina. These results demonstrate that the antigens purified from brain and retina have similar properties. Further work is needed to determine whether the structures of these antigens are indeed identical.

197.7 5'-NUCLEOTIDASE IN SCHWANN CELL PLASMALEMMAE FROM DENERVATED CAT PERIPHERAL NERVE. S.M. Ross*, M.I. Sabri and P.S. Spencer, (SPON: M.I. Sabri). Inst. of Neurotoxicology, Albert Einstein Coll. of Med., Bronx, NY 10461.

Interest in the mechanisms involved in the control of cell-cell interactions has focused attention on surface membrane glycoproteins. A previous study reported isolation and partial characterization of plasma membrane fractions (PM) harvested from an enriched population of undifferentiated Schwann cells resident in denervated cat peripheral nerve (Ross et al., Trans. Am. Soc. Neurochem. 13:267, 1982). This report describes the further purification and characterization of PMs. SDS-PAGE of Schwann-cell PMs revealed the presence of Coomassie-blue-positive diffuse bands (M.W. 60-65K), two PAS-positive bands (M.W. 150-200K), and the absence of myelin-specific proteins. 5'Nucleotidase (5'N), a glycoprotein-dependent enzyme, was enriched in PM preparations. The plant lectin, concanavalin-A (Con-A), reduced 5'N activity in a dose-dependent manner (100-800ug/ml) and, at high concentrations (600ug/ml), activity was inhibited in both crude homogenate (CH) and PM preparations by approximately 80%. Con-A bound specifically to D-mannose and D-glucose moieties of glycoproteins associated with 5'N since activity returned to control levels with pretreatment of the specific hapten sugar, alpha-methyl-D-mannoside (50mM) and to near control values with glucose (50mM). Wheat germ agglutinin (200 ug/ml) also inhibited 5'N activity in CH and PM preparations (55 and 32% of controls, respectively). Preliminary experiments revealed that optimal 5'N activity was obtained at physiological pH (7-8) and at relatively high temperatures (55°C). 5'N activity was magnesium independent (up to 8 mM), although 1 mM EDTA reduced activity to 43 % of control values. Various nucleotides were screened for enzyme specificity and studies indicated that two diphosphorylated nucleotides, UDP and ADP (100uM), reduced 5'N activity to 36 and 23% of PM control values, respectively; these nucleotides thus may serve as substrates for hydrolysis by this enzyme. Results from this study suggest that 5'N is a stable PM enzyme which contains free D-mannose and/or D-glucose residues in glycoproteins resident on the exposed surface that are essential for its activity. The physiological role of 5'N may be of particular importance in neuron-glial interactions since 1) its hydrolysis product, adenosine, is membrane permeant and has been implicated as an intercellular mediator in the nervous system, and 2) exposed glycoproteins essential to 5'N activity may represent an important molecular species involved in cell-cell recognition.

(Supported by NIH grant NS 13106)

197.8 MONOAMINES STIMULATE PHOSPHATIDYLETHANOLAMINE METHYLATION IN RAT BRAIN. C.E. Leprohon*, J.K. Blusztajn*, and R.J. Wurtman. (SPON: T. Maher). Dept. Nutr. & Fd. Sci. MIT, Cambridge, MA 02139.

We have previously shown that mammalian brain synaptosomes synthesize phosphatidylcholine (PC) from phosphatidylethanolamine (PE) by a stepwise methylation catalyzed by phosphatidylethanolamine-N-methyltransferase(s) (PEMT). This newly formed PC can be hydrolyzed to liberate free choline, which may then be available for acetylcholine synthesis.

Catecholamines have been shown to modify the activity of PEMT in a variety of peripheral tissues (Hirata & Axelrod, 1980). However, to date there has been little compelling evidence that the catecholamines are able to stimulate PEMT in central nervous tissue. We now provide evidence that three monoamines, norepinephrine (NE), serotonin (5-HT) and dopamine (DA), enhance the in vitro activity of PEMT in rat brain.

The synaptosomal-enriched P2 pellet from rat brain was resuspended in a buffer (pH=7.5) containing 2.5 μCi (CH_3-^{3}H)-s-adenosylmethionine (15 Ci/mmol) and 100 μM NE, 5-HT or DA. NE, 5-HT and DA increased the incorporation of CH_3-^{3}H groups into the PE methylated products, phosphatidylmonomethylethanolamine (PME) and phosphatidyldimethylethanolamine (PDE) but not into PC. DA was more potent than either NE or 5-HT, with the first methylation (PME) being most sensitive to DA.

pmoles $CH_3{}^3H$ per mg protein per 30 min (mean ± S.D., n=3)

	PME	PDE	PC
Control	0.21±0.02	0.48±0.06	0.16±0.01
NE	0.31±0.01	0.71±0.08	0.16±0.01
5-HT	0.30±0.02	0.67±0.01	0.19±0.01
DA	0.70±0.02	0.69±0.01	0.17±0.01

Osmotic shock and addition of 100 μM ATP and 5 mM $MgCl_2$ to the incubation buffer enhanced the response of PEMT to DA such that a 6-fold increase in CH_3-^{3}H incorporation into PME, and significant increases into both PDE and PC, were observed. The stimulation of PE methylation by DA was completely blocked by co-incubation with 100 μM haliperidol. Phenoxybenzamine and propranolol (100 μM) partially blocked the effect of NE on PEMT. Co-incubation with dibutyryl cyclic AMP or forskolin had no effect on basal or DA-stimulated PE methylation suggesting that adenylate cyclase activation does not modify PEMT activity.

These results suggest that the monoaminergic neurotransmitters may modify the phospholipid composition of synaptic membranes, perhaps mediating some of the slow, nonionic effects of neurotransmission; they may also affect choline availability. (Supported by MH-28783 and Canadian MRC).

197.9 REGIONAL DISTRIBUTION OF PHOSPHATIDYLETHANOLAMINE-N-METHYLTRANSFERASE(S) ACTIVITY IN RAT BRAIN. D. Reinstein*, C. Leprohon*, J.K. Blusztajn* and R. Wurtman. (SPON., E. Spindel) Dept. Nutri. & Food Sci., MIT, Cambridge, MA 02139.

We have previously shown that rat brian synaptosomes contain an enzyme, phosphatidylethanolamine-N-methyltransferase(PEMT), which catalyzes the synthesis of phosphatidylcholine(PC) from phosphatidylethanolamine. This newly formed PC can be hydrolyzed to liberate free choline, possibly for acetylcholine synthesis. We determined whether PEMT was preferentially distributed in cholinergic terminals by simultaneously measuring PEMT and cholineacetyltransferase(CAT) activities in various brain regions.

Incorporation of ^{3}H-methyl groups from S-adenosylmethionine(SAM) into phosphatidylmonomethylethanolamine(PME) was greatest in striatum. ^{3}H-methyl incorporation into PC only showed regional differences when exogenous phosphatidyldimethylethanolamine(PDE) was included in the incubation medium, with pons and cerebellum having the greatest activity. These regional differences in PEMT revealed no simple relationship to CAT activity (see Table).

Transection of the fimbria/fornix decreased hippocampal CAT activity by 80% but had no effect on hippocampal PEMT activity. Together, these data indicate that PeMT is not solely distributed in cholinergic terminals.

We have shown that monoamines, SAM and S-adenosylhomocysteine influence in vitro PEMT activity. Regional differences in levels of these compounds may contribute to the observed distribution of PeMT activity. Therefore, we greatly diluted the concentration of these compounds by osmotically shocking the synaptosomes before PEMT measurement. Osmotic shock had no effect on the regional distribution of PEMT activity, suggesting that regional differences in activity cannot be explained by the presence of endogenous activiators and/or inhibitors. Regional differences in ^{3}H-methyl incorporation into PDE were also observed with the addition of PME to the incubation medium. This pattern followed the same trend as that observed for ^{3}H-methyl incorporation into PC in the presence of PDE.

	^{3}H-methyl incorporation (X±SD) (pmol/mg protein x 30 min.)			(nmol/mg protein x 15')
	PME	PDE	PC	CAT
Striatum	0.54±0.09	1.13±0.17	0.81±0.07	18.8
Cortex	0.31±0.06	1.39±0.05	1.26±0.04	10.9
Hippocampus	0.36±0.04	0.94±0.03	0.89±0.03	14.3
Cerebellum	0.35±0.04	1.88±0.12	2.21±0.04	1.2
Hypothalamus	0.41±0.07	1.11±0.16	0.70±0.07	10.4
Pons	0.50±0.07	3.41±0.07	3.57±0.12	17.3

Thus, while regional differences in PEMT activity exist in rat brain, the significance of this distribution pattern remains unclear. (Supported by MH-28783 and the Canadian MRC).

197.10 FREEZE-FRACTURE ULTRASTRUCTURE OF DEVELOPING AND ADULT RAT RETINAL GANGLION CELL AXON MEMBRANE. J.A. Black*, R.E. Foster, and S.G. Waxman. Dept. of Neurology, Stanford Medical School, VA Medical Center, Stanford, CA and Neurotoxicology and Exp. Therap. Br., U.S. Army Research Institute, Aberdeen Proving Ground, MD.

The intra-retinal segment of the rat retinal ganglion cell axon membrane is non-myelinated in both the neonatal and adult animal. In contrast, the optic nerve segment of this axon is non-myelinated in neonatal rats but fully myelinated in the adult. We previously studied the development of the axolemma during myelination of the optic nerve (Black et al., 1982a). In this study, we examine the freeze-fracture structure of intra-retinal ganglion cell axolemma in developing and adult Long Evans rats in order to determine whether there are any ultrastructural changes in the non-myelinated axolemma during this developmental period.

Retinas from young animals were fixed in situ with 5% glutaraldehyde prior to rapid excision and immersion fixation for 1 hr. Adult retinas were perfused with 5% glutaraldehyde and then immersion fixed for 1 hr. All tissues were then processed according to conventional freeze-fracture methods, and replicas were examined with an electron microscope. Intramembranous particle (IMP) densities (mean±SEM) and sizes (mean±SEM, in nm) for axon membrane E- and P-fracture faces for various ages of retinas are given below:

	E-face		P-face	
Age	IMP/μm^2	size	IMP/μm^2	size
2 day	146±13.7	7.2±0.1	556±42.9	7.2±0.1
8 day	101±11.9	6.9±0.2	510±34.9	6.4±0.1
16 day	112±11.5	7.2±0.1	740±73.1	6.8±0.1
28 day	94±13.5	7.2±0.1	1281±176.9	6.4±0.1
Adult	222±29.5	8.5±0.1	1741±144.5	7.8±0.1

IMPs were generally randomly distributed on both E- and P-faces, except in the optic disc region where linear aggregations of IMPs are observed (Black et al., 1982b). E-face IMP density in the adult is greater than that in developing nerve fiber layer axons; the mean IMP size is also substantially greater in the adult than in the younger animals. On the P-face, there is a gradual increase in IMP density beginning at 8 days of age, with the greatest density in the adult. IMP size is also greatest in the adult. These data demonstrate that the non-myelinated axon membrane of the intra-retinal segment of the ganglion cell displays ultrastructural changes with age. These changes in membrane structure can be compared to the developmental alterations in the myelinated segment of this fiber (optic nerve) and to other developing non-myelinated CNS tracts (Segura-Garcia and Perrelet, 1981). [Supported by the VA, NIH, and Kroc Foundation]

197.11 GLYCOPROTEINS OF CULTURED CHICK CILIARY GANGLION NEURONS. M.Y. Giovanni*, A. Messing, D.E. Pleasure and M.C. Glick.* The Children's Hospital of Philadelphia, Departments of Pediatrics, Neurology, and Pathology and Laboratory Medicine, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

A glycoprotein, M_r=200,000, was found to be associated with excitable membranes in mouse and human neuroblastoma cell lines (Littauer, U.Z., Giovanni, M.Y., and Glick, M.C., J. Biol. Chem. 255:5448, 1980). To determine the extent of this glycoprotein in neuronal systems, dissociated chick ciliary ganglion neurons were examined. These cells provide a primary monolayer culture of non-mammalian species and are an appropriate system to study in vitro neuritic processes which are electrically excitable.

Chick ciliary ganglion neurons from 8-day embryos were cultured relatively free of non-neuronal cells (Messing, A., Brain Research 232:479, 1982) for 6 days and then metabolically labeled for 2 days with L-[^{3}H]fucose or D-[^{3}H]glucosamine as glycoprotein precursors. At 8 days, other cultures were labeled with borotritide for examination of the external glycoproteins. The harvested neurites and cell bodies were examined by 7-14% polyacrylamide gel electrophoresis under denaturing conditions. The radioactive glycoproteins from either of the metabolically labeled cultures showed similar gel profiles. As with the differentiated neuroblastoma cells, a distinct peak was observed in the region of M_r=200,000. Other peaks were seen in the regions of M_r=140,000, 115,000, 90,000, 62,000, 43,000, 30,000 and 17,000. The profile of the externally labeled sialic acid residues showed that most of the fucosylated proteins were also sialylated. The major protein band detected by Coomassie brilliant blue staining was in the region of M_r=50,000, and coincided with a glycoprotein labeled with D-[^{3}H]glucosamine.

The detection of a glycoprotein, M_r=200,000, in cultured chick ciliary ganglion neurons may provide additional support to the correlation of this glycoprotein with the possession of electrically excitable membranes. Supported by NIH Grants HD 08356, HD 07107, NS 07064 and NS 05572.

197.12 GANGLIOSIDE PATTERN CORRELATION WITH METABOLIC RATE IN SALAMANDERS. Louis Irwin and Karen Loehr*. Dept. Biol., Simmons College, 300 The Fenway, Boston, MA 02115.

The functional or adaptive significance of phylogenetic variation in the ganglioside pattern of vertebrate brains remains to be explained, though a prominent theory relates ganglioside composition to thermal habitat (Rahmann H. 1977. Jap. J. Exp. Med. 48:85). We have tested this and the related possibility that ganglioside composition varies with metabolic rate, in the mole salamanders. Adult Ambystoma maculatum were acclimated for 4 weeks to 10°C, 20°C, or fluctuating temperatures that corresponded to those in the natural habitat during June and July in Massachusetts. Metabolic rates of individual salamanders were measured by closed system respirometry; then gangliosides were isolated from whole brain, separated by thin-layer chromatography, and quantified by scanning densitometry. Neither total ganglioside content nor the proportional concentration of the major gangliosides differed significantly among the three treatment groups. However, the relative content of a major ganglioside that comigrated with mammalian GD1a was negatively correlated ($r=-.56$) with individually determined metabolic rates. While the correlation was significant ($p < .01$) when computed without regard to treatment group, it was much stronger in salamanders exposed to fluctuating temperatures ($r=-.99$, $p < .01$) than to those acclimated to 10°C ($r=-.44$, ns) or to 20°C ($r=-.14$, ns). These results suggest that ganglioside patterns may be influenced indirectly by thermal habitat, but that the actual correlation may relate more directly to metabolic rate. (Supported by grants from NIH and the Simmons College Fund for Research.)

197.13 GROWTH OF NERVE FIBERS ON HYDROGEL SUBSTRATES CONTAINING FIBRONECTIN AND GLYCOSAMINOGLYCANS. S.T.Carbonetto and D.C.Turner* Depts of Pharmacology and Biochemstry, SUNY/Upstate Medical Ctr. Syracuse, NY 13210.

Hydrogel substrates prepared from poly-hydroxyethylmethacrylate (HEMA) permit the attachment of neurons cultured from chick embryo dorsal root ganglia but support little nerve fiber growth. Fibronectin added to HEMA before polymerization is trapped almost irreversibly within the hydrogel, rendering it an excellent substrate for nerve fiber growth (Carbonetto et al., Science in press). We report the use of defined HEMA-gel substrates to further characterize the interactions of growing nerve fibers with fibronectin and other components of the extracellular matrix.

Known constitutents of the extracellular matrix of the nervous system include fibronectin, collagen and several glycosaminoglycans. Unlike simple HEMA-gels or HEMA-gels containing fibronectin, HEMA-gels containing the glycosaminoglycans heparin, chondroitin sulfate or hyaluronate do not support nerve cell attachment, let alone fiber growth. Treatment of HEMA-gels containing fibronectin with heparin or incorporation of heparin along with fibronectin in the gel matrix greatly reduces the efficacy of fibronectin as a substrate for nerve fiber growth.

Fibronectin contains distinct functional regions including separate binding sites for glycosaminoglycans, fibronectin, and gelatin and a region which mediates attachment of muscle cells and fibroblasts. Fragments which bear one or more of these functional regions can be isolated following partial proteolysis of native fibronectin. (Ehrismann et al., J. Biol. Chem. 256, 4056,1981). Our preliminary studies with 4 different proteolytic fragments of fibronectin indicate that only those fragments which contain the cell attachment region support nerve fiber growth. Nerve fiber growth on HEMA gels containing 135 and 160 kd fragments of fibronectin was equivalent to growth on HEMA gels with native fibronectin (200kd). By contrast a 93 kd subfragment, lacking the cell attachment site of the 135 kd fragment from which it is derived, promoted little nerve fiber growth when incorporated into HEMA gels.

It appears that growing nerve fibers interact with a select region of fibronectin in the substrate and that this interaction can be modulated by glycosaminoglycans.

197.14 GANGLIOSIDE BIOSYNTHESIS IS INHIBITED BY TUNICAMYCIN IN THE NEUROBLASTOMA X GLIOMA HYBRID NG 108-15. R.L. Schnaar, S.P. Guarnaccia * and J. H. Shaper *. Dept. of Pharmacology, Johns Hopkins University School of Medicine, Baltimore Maryland 21205.

The antibiotic tunicamycin is thought to specifically block the transfer of GlcNAc-1-P from UDP-GlcNac to dolichol phosphate, thereby specifically blocking the synthesis of N-linked oligosaccharide chains on glycoproteins. We have studied the incorporation of [^{3}H]glucosamine into the glycoconjugates of the neuroblastoma x glioma hybrid cell line NG 108-15, and found approximately equal incorporation into glycosphingolipids and glycoproteins. Tunicamycin caused a 78% reduction in incorporation of radiolabelled glucosamine into gangliosides and neutral glycosphingolipids at a concentration which causes a 90% reduction of radiolabel incorporation into glycoproteins. Protein synthesis (measured as [^{3}H]leucine incorporation into TCA-precipitable material) was reduced only 6% under these conditions. The effect of tunicamycin on glycosphingolipid biosynthesis was apparent after 4 hours of incubation, and maximum inhibition was seen within 6 hours. In order to assess the basis for the inhibition, 3 x 10^8 cells were incubated for 3 h with either control medium or medium containing tunicamycin (5 µg/ml), followed by an additional 3 h in the presence of [^{3}H]glucosamine. The cells were collected and fractionated to separate glycoproteins, neutral glycosphingolipids and gangliosides. Nucleotide sugar precursor pools were purified for evalulation of relative specific activities. UDP-GlcNAc and UDP-GalNAc pool sizes increased >3-fold, and specific activities decreased 50% upon treatment with tunicamycin. When corrected for this value, the percent inhibition of glucosamine incorporation into various glycoconjugates by tunicamycin in these cells was: glycoproteins, 82%; neutral glycosphingolipids, 54%; and gangliosides, 50%. Gangliosides were affected differentially, with the most striking inhibition apparent in GM_3 synthesis which was reduced 77% in the presence of tunicamycin. This data demonstrates that tunicamycin has effects on glycoconjugate biosynthesis beyond the inhibition of N-linked glycoprotein glycosylation. While the basis for tunicamycin inhibition of ganglioside and neutral glycosphingolipid biosynthesis is not known, two possibilities are apparent. The antibiotic may directly inhibit the relevant glycolipid glycosyltransferases, or it may block glycosylation of the transferases (which may themselves be glycoproteins) reducing their activities. Whether direct or indirect, the effects of tunicamycin on glycosphingolipid as well as glyocprotein biosynthesis must be taken into account when interpreting effects of this agent on intact cells and organisms. Supported by NIH Grant HD14010, and March of Dimes Grant 5-302.

197.15 CONTROL OF AMILORIDE SENSITIVE NA FLUX IN NEUROBLASTOMA AND GLIOMA CELL LINES. V. Sapirstein and D. Benos (Spon: G. Hauser). E.K. Shriver Center, Waltham, MA. 02254 and Depts. of Biol. Chem. Physiol. and Biophys. and LHRRB Harvard Med. Sch., Boston,MA.02115

We have studied the amiloride sensitive sodium influx into C6 glioma and NB2A neuroblastoma cell lines. In late log phase cells grown continuously in the presence of fetal calf serum showed Na influxes of approximately 20 nmoles/mg protein for both C6 and NB2A cells; < 5% of this was found to be amiloride sensitive. Serum removal for 24 hours did not appear to cause any significant change in this flux, however upon addition of serum to the incubation media there was an increase of 20-40% in the total Na flux within 2 minutes. All of this increment in Na influx was inhibited by amiloride with a $K_{1/2}$ of 50uM. By adding serum back at various times after serum deprivation it was determined that 6 hours was required to observe a detectable increase in the amiloride sensitive Na flux. Addition of 5ug/ml of cycloheximide 4 hours after serum removal was found to block the increase in Na transport indicating induction of de novo protein synthesis mediated this increase in amiloride sensitive Na transport. Moreover, inhibition of de novo lipid synthesis by 0.1 mM fenfluramine blocked the induction of this transport activity suggesting that a coordinated synthesis of lipid and protein is required for the expression of this sodium transport site. The effect of serum removal on Na influx was augmented by the growth of cells in media containing 1.8mM EGTA. Under these conditions NB2A cells displayed a serum stimulated, amiloride sensitive Na influx that was 3 times that of control cells suggesting Ca may influence the expression of the amiloride sensitive Na influx site.

The observation that addition of serum to the media is required to observe the induced increase suggests that the de novo synthesis of membrane components is not sufficient for the functional assembly of this transport site. Since insulin stimulates Na fluxes in several tissues we determined if the rapid effect of serum addition was mediated by this hormone. Experiments indicated that insulin (5m Units) stimulated sodium influx by 72% in NB2A cells; these increments in Na transport however were only slightly inhibited by amiloride (8%). Addition of the calcium ionophore, A23187, or EGTA to the assay media likewise stimulated the Na flux in the serum deprived cells to the same extent as insulin and like this hormone increased only an amiloride insensitive component. The acute effects of insulin, A23187 and EGTA on Na influx may represent the activity of a totally distinct transport site or the same one that was induced by serum removal except lacking the amiloride sensitive component. Studies designed to answer this question are now in progress. Supported by HD05515, NS16186, AM25886 and the Andrew W. Mellon Foundation.

197.16 PHOSPHOLIPID METHYLATION IN MYOGENIC CELL LINES. T.K. Koch*, A.S. Gordon and I.F. Diamond. Univ. of Calif., San Francisco, CA 94143.

The biosynthesis of phosphatidylcholine from successive N-methylation of phosphatidylethanolamine has been implicated as a major mechanism for the transduction of receptor-mediated signals. We have recently developed a rapid and sensitive high resolution method to follow phosphatidyl methyltransferase activity. Lipids are extracted with hexane:isopropanol and the phospholipids separated by high performance liquid chromatography. Using this method, we found an active transmethylation pathway in two differentiated myogenic mouse cell lines, BC3H1 and L8. Addition of carbachol and isoproterenol had no effect on phospholipid methyltransferase activity. However, isoproterenol did cause stimulation of 3'-5' cyclic adenosine monophosphate (cAMP) production in both cell lines. Despite new methodology for a more sensitive analysis of phospholipid composition, no alteration in the transmethylation pathway could be demonstrated with ß-adrenergic stimulation of BC3H1 and L8 even when cAMP stimulation was documented.

197.17 ORTHOGONAL ARRAYS IN MINCED MUSCLE MEMBRANES: PREPARATION VIABILITY, ENERGY DEPENDENCE AND EFFECTS OF CATIONS. James D. Hatton and Mark H. Ellisman. Dept. of Neurosciences, University of California at San Diego, La Jolla, CA 92093.

Orthogonal arrays of particles are known to be plentiful in the sarcolemma of freeze-fractured fast-twitch skeletal muscle, especially so in the sarcospinalis from rabbit. To examine the plasticity of these arrays in muscle membranes, pieces of this muscle were excised from anaesthetized rabbits, minced in 4° C 10 mM tris-buffered .25 M sucrose (pH 7.4), and stored at 4° C for 4, 8 and 24 hr and 2, 3, 4, 5, 6, and 7 days. No change in array number, distribution or morphology was noted at any interval. The addition of 2-50 mM Ca^{++} (as $CaCl_2$) to a similar prep caused the disappearance of arrays by 3 days. When N-ethyl maleimide or phenyl methyl sulfonyl fluoride (PMSF) were added as proteolysis inhibitors, arrays remained intact despite Ca^{++} treatment up to 7 days. Here, however, the addition of 10-50 mM Ca^{++} produced a clustering of whole arrays into patches. Other divalent cations (Mg^{++} and Mn^{++}, as their chloride salts) also induce array disappearance (perhaps through proteolysis) and produce array-clustering, though to a lesser extent than does Ca^{++}. These effects are not noted with the addition of 10-50 mM NaCl, KCl or choline chloride, suggesting that array-clustering is neither dependent on univalent cations nor on Cl^- ion per se.

In similar experiments, minced muscle was incubated for 3 days at 4° C in the presence of PMSF. It was found that the addition of 10-100 mM adenosine triphosphate (ATP) increased the total number of arrays, but did not induce clustering. Meanwhile, the addition of 10-50 mM dinitrophenol reduced the number of arrays, to nearly zero in the case of the highest concentration. Ouabain ($5X10^{-4}$ M) alone produced no effect on array density, but did inhibit the ATP-induced array proliferation. This suggests that (at least) the appearance of orthogonal arrays in the membrane may be related to Na^+-K^+ ATPase activity. Finally, the addition of 10-50 mM dibutyryl c-AMP produced neither clustering nor an increase in array density, suggesting that this putative second messenger is not involved in either of these effects.

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197.18 EFFECTS OF SAPONIN ON ACETYLCHOLINE RECEPTOR CLUSTERS OF CULTURED RAT MYOTUBES VISUALIZED BY FREEZE FRACTURE. D.W. Pumplin* and R.J. Bloch, Anat. and Physiol. Depts., Univ. of Maryland Sch. of Medicine, Baltimore, Md. 21201.

Clusters of acetylcholine receptors (AChRs) in substrate-apposed membrane of cultured rat myotubes are often organized linearly: regions of AChR enrichment ("AChR domains") interdigitate with sites of closest approach of the membrane to the substrate ("contact domains") [1]. To learn if lipids are heterogeneously distributed in these two membrane domains, we studied the effects of the cholesterol-specific detergent, saponin, on AChR clusters using freeze fracture electron microscopy.

Rat myotubes cultured on glass cover slips were exposed at 22° to 0.2% saponin in a modified buffered saline for 15 sec to 8 min then fixed in 2% glutaraldehyde. Circles cut from the cover slips were freeze-fractured using the complementary replica method [2]. Replicas from the specimen carrier side consisted almost entirely of P faces from substrate-apposed membranes of myotubes and fibroblasts.

The overall linear organization of AChR clusters was not disturbed by treatment with saponin. AChR domains, containing the large intramembrane particles (IMPs) corresponding to AChR [2], remain distinct from contact domains. Within the AChR domains the following changes were seen after 1 min of saponin exposure: formation of shallow, poorly defined depressions; aggregation of the large IMPs; and large scale (>100nm) deformations of the membrane. In contrast to these changes in the AChR domains, the contact domains were almost unaffected by saponin: they showed little membrane deformation and no IMP aggregation. Myotube membrane outside of the clusters was likewise unaffected.

In fibroblasts, the following changes were seen: (i) at 30-60 sec, individual, sharply defined pits (44nm diam) formed; (ii) ≥1 min, large pits aggregated; (iii) ≥3 min, IMPs tended to aggregate into membrane surrounding the pit aggregates. These changes, and those seen in myotubes, were blocked by prefixation.

Saponin not only affects myotubes and fibroblasts differently, it also distinguishes between the AChR and contact domains within AChR clusters. This can be interpreted in two ways. (i) Cholesterol may be enriched in AChR domains, or may become so upon exposure to saponin. (ii) Cholesterol is homogeneously distributed between AChR and contact domains, but only the AChR domain can deform enough to reveal the effects of saponin-cholesterol complex formation.

[1] Bloch, R.J. and Geiger, B. (1980) Cell 21, 25.
[2] Cohen, S.A. and Pumplin, D.W. (1979) J. Cell Biol. 82, 494.
Supported by NIH grants NS 15513 and NS 17282 and by grants from the Muscular Dystrophy Association.

197.19 DEVELOPMENTAL REGULATION OF THE APPEARANCE OF SYNAPTIC BASAL LAMINA ANTIGENS IN ANEURAL MUSCLE CULTURES. L. Silberstein* and Z.W. Hall. Dept. of Physiology, U. Calif. Sch. of Med., San Francisco, CA 94143.

The basal lamina (BL) that runs between the presynaptic and postsynaptic membranes at the adult neuromuscular junction has distinctive functional properties (1,2,3) and may be distinguished immunologically from the extrasynaptic BL to which it is attached (4). In regenerating muscle, the synapse-specific accumulation of acetylcholine receptors occurs precisely at the old endplate, even in the absence of a neural partner, at the site marked by the synpatic BL formed prior to injury (2,3). This observation suggests a specific interaction between components of the extracellular matrix and acetylcholine receptors (AChR) clusters.

We have previously reported that myotubes of a mouse muscle cell line, C_2, have surface accumulations of BL antigens that are recognized by each of three synapse-specific antisera (5). We report here experiments in which we have studied quantitatively the developmental expression of two of the synaptic BL antigens (JS-I and JS-II), and the progressive association of one of them with AChR clusters.

A radioimmunoassay was used to measure the amount of the JS-I and JS-II antigens in C_2 cultures. Both were present at very low, or undetectable, levels in myoblasts and began to increase within 24 hours after myoblast fusion began. Over the next four days, both continued to increase approximately tenfold. The overall time course of expression of the BL antigens resembles that seen for the accumulation of acetylcholinesterase (AChE) or AChRs in myotubes after fusion.

One of the antigens (JS-I) is often found in close association with AChR clusters. The degree of this association increases with time in culture. Thus, in myotubes examined two days after fusion has begun, approximately 50% of AChR clusters are associated with JS-I patches, while two days later, over 95% of the clusters are so associated. Over the same time period, a comparable increase in the percentage of patches associated with AChR clusters occurred Furthermore, the density of JS-I antigens at AChR patches was noticeably higher in later cultures. This observation is consistent with the increase seen by radioimmunoassay. This system may provide a useful in vitro model for understanding the specific association in vivo between AChR clusters and synaptic BL.

This work was supported by grants from the NIH, Muscular Dystrophy Foundation, and the National Science Foundation.

(1) Sanes et al. (1978) J. Cell Biol.78:176; (2) Burden et al. (1979) J. Cell Biol. 82:412; (3) Bader (1981) J. Cell Biol. 88: 338; (4) Sanes and Hall (1979) J. Cell Biol. 83:357; (5) Silberstein, Inestrosa and Hall (1982) Nature 295:5845.

197.20 AUTORADIOGRAPHIC STUDIES OF α-BUNGAROTOXIN BINDING IN HIPPOCAMPAL CULTURES. G.A. Banker and J.E. Mazurkiewicz*. Department of Anatomy, Albany Medical College, Albany, New York, 12208.

We have studied the development and localization of binding sites for α-bungarotoxin in hippocampal cell cultures prepared from the brains of fetal rats. Two types of cultures have been examined. Some were prepared at high cell densities (15,000 cells/cm^2), which permit the cells to become extensively innervated; others were prepared at low plating densities (1,500 cells/cm^2), so that some cells develop without contacting any other neurons or glia. After varying periods of development in vitro, the cultures were incubated with (^{125}I)-α-bungarotoxin (1 to 6 X 10^{-9} M) for 1 hour, then fixed and processed for light microscopic autoradiography. Non-specific binding was assessed in control cultures treated with excess unlabeled toxin or with tubocurare (10^{-4} M) before exposure to (^{125}I)-α-bungarotoxin.

Specific binding sites for α-bungarotoxin were not detectable on the first day in culture, but appeared within the first week. Thereafter their density increased rapidly, becoming maximal by two to three weeks. This roughly parallels the increase in binding seen during hippocampal development in vivo. After two to three weeks in culture about two-thirds of the neurons were labeled by this method. No specific labeling of non-neuronal cells was observed.

After two weeks in culture binding sites were present on cell somata and along the cells' dendritic processes. Within the resolution of this method, the binding sites appeared to be uniformly distributed over these regions rather than clustered in patches. Binding sites were not detected on the cells' axons, which are thinner in diameter than the dendrites. The pattern of labeling was the same on uninnervated, completely isolated cells as it was on cells in dense cultures which received heavy synaptic input. Thus the acquisition of binding sites for α-bungarotoxin during neuronal development can occur independent of innervation.

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197.21 CO-LOCALIZATION OF ACh RECEPTOR CLUSTERS AND NUCLEI IN CULTURED MYOTUBES. Libbe L. Englander* and Lee L. Rubin. The Rockefeller University, New York, N.Y. 10021.

During nerve-muscle synapse formation, some acetylcholine receptors appear to redistribute from extrasynaptic to synaptic sites. There is evidence, however, to suggest that the synaptic cluster is subsequently maintained by local receptor insertion. We have begun to examine how the cell might be organized for this local insertion of membrane components by studying the formation of acetylcholine receptor clusters on cultured chick myotubes induced by factors derived from Torpedo electric organ extracellular matrix. We have now examined in detail the relative positions of clusters and myotube nuclei 36 hours after treating cultures with these factors.

The acetylcholine receptor clusters were visualized with rhodamine α-bungarotoxin; the nuclei were stained with the fluorescent dye 4',6-diamindino-2-phenylindol-2HCl. Photomicrographs of 10 cells (a total of 78 fields) were used to make measurements of the area and position of each cell, its nuclei and its clusters. On the average, 50% of the 548 clusters counted were within 5-6 microns of their nearest nucleus although the average distance between nuclei is 36.8 microns. To establish firmly an association between cluster and nuclear location, we calculated the mean distance of clusters from their nearest nucleus in each cell and the expected mean nearest distance and standard deviation under the null hypothesis that clusters were randomly placed relative to nuclei. The test yielded a χ^2 of 76.24 ($p<.001$), emphatically suggesting an association between the nuclei and clusters.

Such an association could develop if clusters formed preferentially over nuclei. It is also possible, however, that clusters first form randomly and then induce nuclei to localize beneath them; we are examining the latter possibility. In addition, we are determining the position of other intracellular organelles, such as the Golgi apparatus, to establish whether they are located preferentially near the cluster-related nuclei.

197.22 COMBINING RECEPTOR AUTORADIOGRAPHY AND IMMUNOCYTOCHEMISTRY: AN ANALYSIS OF β-ADRENERGIC RECEPTORS ON GFAP+ ASTROGLIA. Ken D. McCarthy* (SPON: Susan K. Burgess). Dept. of Pharmacology, Univ. of North Carolina, School of Medicine, Chapel Hill, NC 27514.

A number of studies indicate that cultured astroglia isolated from neonatal rat or mouse brain possess a variety of receptors which regulate cyclic AMP accumulation. However, the issue of whether or not such receptors are characteristic of mature astrocytes has not been resolved. This is due, in part, to the difficulty in isolating mature astrocytes in sufficient number to complete receptor analyses via standard radioligand binding techniques. The research described here concerns a novel method which can be used to monitor the density of β-adrenergic binding sites on single cells which have been identified via immunocytochemical methods.

Primary cultures containing predominatly immature astroglia and oligodendroglia and a few morphologically mature astrocytes were harvested from brain cell cultures, seeded onto polylysine coated glass slides and incubated for 2-7 days prior to analysis. The cells were then rinsed, aldehyde fixed, and permeabilized to permit the intracellular staining of the astroglial cell marker, glial fibrillary acidic protein (GFAP). The cells were then incubated in culture medium (BME) containing rabbit antiserum to GFAP, rinsed and incubated with BME containing rhodamine conjugated goat anti-rabbit antiserum and (-) (^{125}I)-iodopindolol (^{125}I-PIN). The cells were then rinsed in PBS containing propranolol, frozen, lyophilized, and opposed to emulsion coated coverslips. After 2-3 weeks the autoradiograms were developed and the grains over GFAP+ cells visualized via reflected polarized light microscopy. The number of silver grains per μm^2 over cells and background was quantified by an Apple II+ computer interfaced with a digitizer.

Extensive biochemical binding assays have been completed to examine the influence of the methods described above on receptor binding characteristics. These experiments indicate that the methods described above do not alter 1) the rate of association of ^{125}I-PIN to cells, 2) the rate of dissociation of ^{125}I-PIN from cells, 3) the number of ^{125}I-PIN binding sites per cell, 4) the K_D of ^{125}I-PIN, or 5) the stereospecific displacement of ^{125}I-PIN by (-) propranolol and (+) propranolol. However, one consistent effect resulting from fixation was an increase (10-100 fold) in the potency of both (-) and (+) isoproterenol to displace ^{125}I-PIN from binding sites. This effect appears to be due to the ability of an agonist, such as isoproterenol, to induce a rapid decrease in the affinity of β-adrenergic receptors for agonists on intact, nonfixed cells.

Preliminary results using the method described above suggest that there can be more than a 10-fold difference in the number of ^{125}I-PIN binding sites amoung GFAP+ cells. Experiments in progress are designed to correlate the degree of morphological differentiation of GFAP+ cells with the number of ^{125}I-PIN binding sites per cell. Supported by NS16992 and NS16229.

197.23 EVIDENCE FOR FUNCTIONAL A_1 AND A_2 ADENOSINE RECEPTORS ON HAMSTER AND HUMAN SPERMATOZOA. B.E. Morton, M. Thenawidjaja* and S. Dimsdale*. Department of Biochemistry and Biophysics, University of Hawaii School of Medicine, Honolulu, Hawaii, 96822.

The presence of adenosine receptors upon hamster caudal epididymal (HCE) spermatozoa was observed. Evidence for this discovery included data of the binding to HCE sperm of N^6-cyclohexyladenosine (CHA), Bmax 2.87 pmoles/10^{10} cells, Kd 12.9 nM; and the binding of 1,3-diethyl-8-phenylxanthine (DPX), Bmax 7.7 pmoles/10^{10} cells, Kd 19.8 nM. [^{3}H]-CHA was displaced from HCE sperm by a series of nucleosides and purine bases in order quite different than that observed for [^{3}H]-DPX and in a manner consistent with the presence of both A_1 and A_2 classes of adenosine receptors on these cells. The A_1 adenosine receptor had been found only in brain and testes. On a unit weight basis the Bmax of CHA for HCE sperm exceeds that of testes by 10 fold, suggesting that the testicular A_1 adenosine receptor may reside entirely upon spermatozoa.

Spermatozoa may be valuable for adenosine receptor studies not only because, unlike other tissue, each cell carries receptors, but also because the practical consequences of receptor occupancy manifest themselves in terms of sperm motility. Thus, CHA inhibited and L-PIA moderately stimulated HCE sperm motility at concentrations approaching one molecule/cell while at μM concentration the effects of these nucleosides agonists were reversed. The antagonist, caffeine, totally overrode these effects to cause its well-known powerful stimulation of motility. Another antagonist, DPX, had no effect upon sperm motility by itself but potently blocked all effects of CHA. Further consequence of receptor occupancy detected was the stimulation of [^{45}Ca] efflux from HCE spermatozoa by very low concentrations of CHA. Adenosine receptors were also demonstrated to be present on human spermatozoa.

The biological significance of adenosine receptors upon mammalian spermatozoa is potentially great. Although evidence suggesting the presence of excitatory neuroreceptors for catecholamines, acetylcholine and taurine exists, the discovery of inhibitory (A_1) adenosine receptors presents a means whereby the female may retard inward sperm migration and in certain cases coordinate it with ovulation. The agitated motility of capacitated spermatozoa, appearing several hours after their entry into the female, would now appear to represent their release from inhibitory restraint prior to concerted migration to the ovum.

It is therefore possible that endogenous A_1 and A_2 ligands exist not only in female reproductive tract but also in that of the male of those species whose motility is suppressed until ejaculation. These systems may have contraceptive potential.

198.1 SUSCEPTIBILITY OF THE SQUIRREL MONKEY TO SEVERAL DIFFERENT MOTION CONDITIONS. R.A. Fox*, N.G. Daunton and J. Coleman*. San Jose St. Univ. and NASA-Ames Research Center, Moffett Field, CA, 94035.

The exact stimulus eliciting vomiting in animal studies of motion sickness is difficult to specify because the vestibular stimulation produced by many motion conditions is confounded by voluntary movements by the animals. This is an important problem because experiments with animal models of motion sickness can provide useful information about antimotion sickness drugs or the role of neural mechanisms, only when animals are exposed to the same motion stimuli in each experimental session.

A series of tests were conducted to determine the susceptibility of 15 adult squirrel monkeys to motion sickness in freely moving and restrained test conditions. Canal stimulation was varied by exposing the monkeys in freely moving conditions to varying degrees of angular velocity (60,90,120,150 deg/sec), and in restrained conditions to one angular velocity (150 deg/sec) and to cross-coupling effects of whole-body roll movements during rotation. Otolith stimulation was investigated by using sinusoidal vertical linear acceleration during free movement conditions, and off-vertical rotation and earth-horizontal (BBQ) rotation while restrained.

The percentage of freely moving animals vomiting during vertical axis rotation was 27, 93, 86, and 92 for the angular velocities of 60, 90, 120, and 150 deg/sec respectively. None of the monkeys vomited during vertical axis rotation or cross-coupled rotation when restrained. Otolith stimulation appears to be a less provocative stimulus for the squirrel monkey as the percentage of animals vomiting were 13, 0, and 7 for the conditions of free movement during oscillation, restraint during off-vertical and BBQ rotation respectively.

Motion sickness to the point of vomiting occurred regularly only in conditions where self-motion was possible. Such effects could occur because voluntary movement during motion augments vestibular effects by producing self-inflicted cross-coupling, but the failure to elicit vomiting with experimenter-produced cross-coupling argues against this interpretation. Alternatively, these results might imply that feedback from movement control mechanisms may play an important role in sensory conflict as suggested by Oman's sensory-motor conflict theory.

198.2 EFFECT OF VESTIBULOSPINAL TRACT LESIONS ON CAT NECK AND FORELIMB TILT REFLEXES. A.D. Miller, P.S. Roossin*, R.H. Schor and V.J. Wilson. Rockefeller University, New York, NY 10021

Tilt about the longitudinal (roll) axis of decerebrate cats produces extension of the side-down forelimb and contraction of side-up neck extensors. The otolith organs form a major input to this reflex; a semicircular canal contribution at tilt frequencies above 0.1 Hz is required to maintain a compensatory response. In cats whose semicircular canals have been inactivated, only vestibular neurons excited by side-up static tilt exhibit muscle-like reflex dynamics (Schor, Miller and Wilson, Soc Neurosci Abst 7: 690, 1981), suggesting that these neurons might contribute to the reflex observed in the ipsilateral neck and contralateral limb.

The present experiments examined the role of the medial (MVST) and lateral (LVST) vestibulospinal tracts in the production of roll tilt reflexes, in decerebrate cats with intact labyrinths. Sectioning the medial longitudinal fasciculus, which contains the MVST, had no major effect on the phase of the reflex EMG recorded from the forelimb extensor triceps brachii and the neck extensors splenius and biventer cervicis, although some gain was usually lost at high stimulus frequencies. Spinal lesions at C_2-C_3, both cord hemisections and smaller unilateral lesions restricted to the area of the LVST, produced two major effects on the forelimb. Background EMG activity was usually abolished in the triceps ipsilateral to the lesion, with some loss of activity in the opposite limb. The tilt reflex response in the ipsilateral limb appeared normal, although it was usually necessary to raise the background excitability of the preparation by administering L-Dopa (50 mg/kg i.v.) in order to observe the reflex. In contrast, the response in the limb contralateral to the lesion showed a phase reversal of 180 degrees at low stimulus frequencies, implying that the reflex in intact cats receives a crossed otolith-spinal input. Responses in the neck extensors splenius and biventer, recorded from compartments caudal to the spinal lesion, were relatively unaffected.

These results demonstrate that neither the MVST nor LVST is essential for the production of roll tilt reflexes in the neck. The reflex pathway to forelimb extensors has a crossed otolith-spinal component, which probably involves the LVST. (Supported in part by NASA grant NSG-2380, PHS grants NS02619 and RR07065, NIH Postdoctoral Fellowship NS06128, and NIH Research Service Award 7524).

198.3 ANTIMUSCARINIC AND SOMATOSTATIN-INDUCED BARREL ROTATION ARE ELICITED BY MICROINJECTION OF VESTIBULAR NUCLEAR COMPLEX. R.E. Burke & S. Fahn, Department of Neurology, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

Barrel rotation (BR) is a behavior observed in rats which consists of sustained extension of the limbs on one side of the body and torsion of the trunk about the long axis towards the opposite side, resulting in repetitive lateral rolling. This phenomenon was first described following intraventricular injection of somatostatin (SRIF) (Cohn & Cohn, Brain Res., 1975), and it has since been observed following injection of vasopressin, Substance P and other neuropeptides. Biologically inactive analogues of SRIF do not induce BR. We have reported that "experimental dystonia" in rats induced by intraventricular injection of chlorpromazine-methiodide (CPZMI) is identical in appearance to SRIF-induced BR, and that it is an antimuscarinic effect. The purpose of the present investigation was to determine the site of action of SRIF and antimuscarinics to elicit BR when injected intraventricularly. Our approach was to study dose-response for microinjections of these compounds to elicit BR with changes in placement of intraventricular and then intracerebral cannula positions.

We found that right lateral ventricle injections of increasing doses of CPZMI or SRIF in 3 µl failed to elicit BR. Dye studies of such injections showed distribution to R and L lateral and IIIrd ventricles. In contrast, caudal IVth ventricle injections of small doses of either compound in 3 µl promptly elicited BR. Dye studies of these injections distributed to IVth ventricle and Aqueduct. We concluded that IVth ventricle injection of these compounds is necessary and sufficient to elicit BR. Control injections of striatum, cerebral cortex, and cerebellar deep nuclei failed to elicit BR. Excluding cranial nerve nuclei, cholinergic receptors adjacent to the IVth ventricle are located largely in locus coeruleus (and other surrounding nuclei) and the medial vestibular nucleus (Wamsley et al., J. Neurosci., 1981). We found that CPZMI 10 nmole and SRIF 1 nmole in 0.5 µl failed to elicit BR when injected unilaterally into the locus coeruleus, but elicited BR promptly when injected unilaterally into the vestibular nuclei. In addition, whereas the direction of BR could be right or left following intraventricular CPZMI or SRIF injections, it was always to right following right vestibular nuclei injection.

Thus, dose-response studies of these microinjections suggest that antimuscarinics and SRIF act at the vestibular nuclei to elicit BR when injected intraventricularly. This conclusion is supported by the behavioral observation that, following labyrinthectomy, vertebrates develop limb extension and torsion about the long axis with lateral rolling, as seen in BR.

198.4 THE EFFECT OF ACOUSTIC TUMOR SIZE AND LOCATION ON OCULAR COUNTERROLLING. Shirley G. Diamond and Charles H. Markham. Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Dynamic ocular counterrolling (OCR) testing was performed on patients with tumors in the internal auditory canal and/or cerebellopontine angle. Their responses to constant velocity roll about their naso-occipital axes were compared to responses obtained from normal subjects.

The test protocol consisted of patients being tilted to 90° right ear down at a constant velocity of 3°/sec, held there for 30 sec, rolled in the opposite direction to 90° left ear down, held there 30 sec, and brought back through the upright position. This was termed Trial I. Without stopping, the procedure was repeated for Trial II. Acceleration and deceleration during the first and last 20° of rotation was 0.21°/sec², considered to be below the threshold of the semicircular canals. Photographs were taken at each 10° of roll.

Measurements of OCR were made with a two-projector apparatus using a superimposition technique. This system is accurate to 0.25°. Each eye was measured independently.

Measurements were plotted on forms preprinted with the norms derived from the means and standard deviations of our group of normal control subjects.

Analysis of OCR profiles of patients with diagnosed tumors of various sizes revealed three distinct groups: 1) those with OCR profiles almost normal in appearance or with mildly abnormal responses to tilt to the side opposite the tumor; 2) moderately abnormal to both directions of tilt, more so to the side opposite the tumor; 3) severe derangement of the entire pattern of response with the eyes rolling instead of counterrolling to the appropriate direction of tilt.

These types of OCR abnormalities corresponded to the size and location of the tumors and the extent to which the tumor impinged on the utricular nerve and brainstem. The slightly disturbed profiles were seen in patients with small (< 1 cm) intracanalicular tumors. If the tumor completely filled the internal auditory canal and extended into the cerebellopontine angle, the OCR profile showed greater disorganization. If the tumor also caused major compression of the brainstem, OCR profiles showed profound disturbance which persisted in testing several months after surgery. This implies that a combination of utricular and brainstem involvement may lead to a permanent disruption of pathways subserving the OCR response.

198.5 MECHANISMS OF AMPHIBIOUS ACCOMMODATION IN TURTLES. D. P. M. Northmore and A. M. Granda. Institute for Neuroscience, Univ. of Delaware, Newark, DE 19711.

Adaptations to amphibious vision were studied in the freshwater pond turtle, Pseudemys, and in the wholly marine Chelonia. The focal length of the anterior half of one eye of each specimen was measured. The other eye was frozen, sectioned and photographed to determine its dimensions. Combining these measurements gave estimates of the bulk refractive indices of the lenses. Schematic eyes were then drawn depicting the unaccommodated eye of Pseudemys as emmetropic in air and 90D in water; that of Chelonia as 10D myopic in air and 30D hyperopic in water. These figures agreed with retinoscopic measurements of refractive error. Accommodation was studied by electrical stimulation of the anterior half of the eye while measuring the radii of lens curvature with Purkinje images. The latter showed that the power of the lens was increased almost entirely by deformation of its anterior surface, accomplished largely by a squeezing action of the annular pad by the ciliary muscle. An additional role was played by the constriction of the iris. In both species, the range of accommodation was more than sufficient to neutralize their hyperopia underwater. Surprisingly, the Chelonian eye at rest requires little correction in air, although as expected, it needs less correction underwater than does the eye of Pseudemys.

198.6 PROJECTIONS TO THE THALAMUS FROM BRAINSTEM RETICULAR AREAS AND VESTIBULAR NUCLEI IN THE RAT. G.A. Kevetter, W.R. Mehler, & W.D. Willis; Marine Biomed. Inst., Dept. of Anat., Physiol. Biophys. & Otolaryn.. Univ. Texas Med. Br., Galveston, TX 77550 and NASA - Ames Res. Ctr., Moffett Field, CA 94035

Cells in the brainstem that project to the thalamus were studied in the rat. In order to label as many cells as possible, large, multiple, unilateral injections of horseradish peroxidase and fluorescent dyes (DAPI and nuclear yellow) were made into the caudal half of the thalamus. Our intention was to include both intralaminar and principal thalamic nuclei. With the exception of the dorsal raphe nucleus labeled cells were located bilaterally. Cells found in a given area, however, were concentrated either ipsilateral or contralateral to the side of injection. The largest number of labeled cells were found consistently in the contralateral dorsal column nuclei and the spinal and principal trigeminal nuclei. In the medulla other labeled cells also were found chiefly contralateral to the side of the injections. A strikingly dense group of labeled cells were found along the lateral border of the contralateral descending vestibular nucleus in 4 out of 5 of the initial experiments. Sporadic cell labeling appeared in the other vestibular complex nuclei, especially in a Z-like group dorsal to the medial nucleus and throughout nucleus prepositus hypoglossi and paramedian reticular formation regions. At pontine and mesencephalic levels ipsilateral cell labeling predominated. The ipsilateral nucleus pontis oralis, locus coeruleus, dorsolateralis tegmenti and the lateral wing of the dorsal raphe nuclei exhibited moderate numbers of labeled cells. Some cells frequently appeared in the ipsilateral ventrolateral mesencephalic tegmentum and in a dorsolateral tegmental cell group. (Supported by NIH grants 09743 and 11255, training grant NS 05743 and a grant from the Moody Foundation).

198.7 THE CYTOARCHITECTURE AND SACCULAR INNERVATION OF NEURONS WITHIN NUCLEUS Y OF THE MOUSE. D.R. Trune and C.J. Frederickson. Dept. Otolaryngology, Ohio State University, Columbus, OH 43210 and Dept. Psychology, University of Texas at Dallas, Richardson, TX 75080.

Our laboratories have been independently studying nucleus y in the normal mouse to determine its boundaries, neuronal forms, and saccular afferents as the normative basis for its subsequent examination after sensory deprivation in otoconia-deficient mutant mice (Smith et al, Soc. Neurosci. Abstr., 7:481, 1981; Trune and Lim, Assoc. Res. Otolaryngol. Abstr., p. 61, 1982). Our observations of Nissl- and Golgi-stained material have revealed two predominant (though not exclusive) neuronal types within this central vestibular cell group.

The most common cytological type within nucleus y is the fusiform cell. In Golgi-Cox impregnated material, the soma of this neuron is fusiform or cylindrical in shape and gives rise to one or two dendrites at each of its poles. These dendrites often branch secondarily but the entire dendritic field usually remains within the boundaries of nucleus y. These neurons in Nissl-stained tissue also appear somewhat fusiform or oblong in shape, with the length (12-20 μm) about 1 1/2 times the width (8-15 μm). The Nissl substance is more prominent at the poles of the soma and thin or absent on either side of the nucleus. These neurons occur throughout the nucleus although they are less frequent in its caudal region.

The second dominant cell type is the stellate cell. Golgi-Cox impregnation of these reveals 4-6 dendrites emanating from the spherical cell body. These dendritic fields are mostly confined to nucleus y as well. In Nissl stains, their somas are spherical, range in diameter from 12-25 μm, and have a distinct, complete ring of Nissl substance surrounding the nucleus. These neurons also occur throughout nucleus y, but the largest of these cells are most common in the caudalmost region.

Microinjections of horseradish peroxidase into the saccule, labeling 4-12 axons, reveal that the saccular afferents terminate in the immediate vicinity of both the fusiform and stellate cells. Although counterstaining shows most terminals do not contact the perikarya, some terminals are found closely apposed to somas or dendritic stumps. This latter pattern of apparent synaptic contact occurs on both the stellate and fusiform cell types.

198.8 **CENTRAL PROJECTIONS OF THE HORIZONTAL AMPULLARY NERVE OF THE GUITARFISH, RHINOBATOS PRODUCTUS (CHRONDRICHTHYES: BATOIDEA). D.M. Koester, R.F. Dunn. Div. Vest. Disorders, Dept. Otol., Univ. of Pittsburgh, Sch. Med., Eye & Ear Hosp., Pittsburgh, PA 15213.**

The central projections of first order horizontal ampullary nerve (HAN) afferents of the guitarfish were elucidated by the anterograde transport of HRP. Anesthesia used for the surgical procedures and perfusion consisted of a controlled mixture of halothane, oxygen, and nitrous oxide bubbled through seawater. The HAN was exposed dorsally on one side and transected distal to the octavus ganglion at the level where the HAN enters the crista. HRP crystals were applied to the proximal stump of the HAN for 25-30 minutes. Prior to closure of the exposed area, the nerve was rinsed with distilled water to prevent diffusion of HRP within the otic capsule. Two weeks postoperatively, the guitarfish were perfused and transverse sections of the brain stems and horizontal sections of the octavus nerve were reacted with TMB.

Anterograde transport of HRP reveals labeled first order HAN afferents that enter the medulla and bifurcate to give rise to ascending and descending pathways within discrete regions of the ipsilateral octavus nuclear column. The ascending fibers are confined primarily to the ventrolateral portion of nucleus octavus anterior (NOA). Rostral to the level of entrance of the Vth cranial nerve, several ascending fibers course dorsally from NOA and project to the lateral and medial granular layers and lower lip of the vestibulolateral lobe of the cerebellum. Fibers of the descending pathway are confined predominantly to the ventral portion of nucleus octavus descendens (NOD) and nucleus octavus posterior. Throughout the rostrocaudal extent of the NOD, several descending fibers and/or collaterals exit its ventromedial border and form a broad fiber tract that projects to a small nuclear region ventral to the reticular formation. Some of these fibers project and presumably terminate within the reticular formation. In addition, a few descending fibers emanate from the dorsal border of NOD and course through nucleus intermedius. The termination site of these fibers is uncertain although it appears they project to the Purkinje-like cell layer of the posterior lateral line lobe.

Primary afferent projections to nucleus magnocellularis are uncertain as the axons of the efferent components of the HAN course through this nucleus to exit the brain via the octavus nerve root.

Retrograde transport of HRP reveals several labeled fusiform cells, both ipsilaterally and contralaterally, within a longitudinal column that is located immediately beneath the fourth ventricle, dorsal to Steida's fascicle and extends from the level of entrance of the octavus nerve to the rostral border of the visceral motor column. These cells are presumed to be the cells of origin of the efferent components of the HAN.

Supported by a grant from the Deafness Research Foundation.

198.9 IDENTIFICATION AND LOCALIZATION OF GUITARFISH OCTAVUS NUCLEI BY NERVE DEGENERATION. R.F. Dunn and D.M. Koester. Div. Vestibular Disorders, Dept. Otolaryngology, Univ. Pittsburgh Sch. Med., Eye & Ear Hospital, Pittsburgh, PA 15213.

Previous studies have shown that different regions of the horizontal semicircular canal crista initiate distinctive impulse response functions which are transmitted to the CNS through anatomically separated nerves. Functional distinctions between the horizontal canal and the two vertical canal ampullary nerves have also been demonstrated. Therefore, identification and mapping of the central octavus nuclei are of interest so that the central distribution of the first order afferent neurons may be determined. The octavus nuclei were identified from transverse-sectioned brains of Rhinobatos productus and R. lentigenosus stained with cresylviolet, or Fink-Heimer I following unilateral transection of the octavus nerve proximal to its ganglion.

Results from the transection studies indicated octavus nerve degeneration primarily confined to four nuclei located along the ventral column within the octavolateralis region. Rostrocaudally the nuclei were: nucleus octavus anterior (NOA); nucleus magnocellularis (NM); nucleus octavus descendens (NOD); and nucleus octavus posterior (NOP). The columnar distribution of the nuclei became readily apparent when mapped onto either a midsagittal plane or onto a ventral plane. Whereas the projections were prepared section by section from serial sections, the resulting map was analogous to outlining a shadow of the nucleus projected onto either plane. The transition between the NOP and NOD appeared rather abrupt, separated by only a narrow region which could easily be missed without mapping. The area between the NOD and NOA was readily defined by the entrance of the lateral line-octavus-VII complex where these nerve fibers sweep into the CNS. The large cells of NM were easily identified in the cresylviolet stained sections at the level where the octavus nerve enters the CNS. Degenerating nerves, also observed at this level, coursed ventrally towards a small nucleus near the reticular formation. Whether or not these fibers were descending fiber projections, or possibly collaterals, remains uncertain. The cellular components within the octavus nuclei in both species appeared similar to each other and to that described in other skates.

Confinement of degenerating first order octavus afferents to the ipsilateral octavus nuclei was similar to octavus projections reported for other elasmobranchs. The distribution of primary afferent neurons within the octavus nuclei, together with the cytoarchitectural identification and spatial localization, are prerequisite for determining to what extent the anatomical separation established in the peripheral end organs is maintained centrally.

Supported by a grant from the Deafness Research Foundation.

198.10 MOTION-INDUCED ALTERATIONS IN 2-DEOXYGLUCOSE UPTAKE IN BRAIN STEM NUCLEI OF SQUIRREL MONKEYS AS DEMONSTRATED BY SEQUENTIAL DOUBLE-LABEL METHOD. K. R. Brizzee and W. Dunlap*. Dept. Neurobiology, Delta Regional Primate Res. Ctr., Covington, LA 70433 and Dept. Psychology, Tulane Univ., New Orleans, LA 70118.

Six young adult squirrel monkeys were injected intravenously with 167μCi/100 gram body weight of (1-^{3}H)-2-deoxy-D-glucose (New England Nuclear, 7.2 Ci/mmole) in 0.5 ml sterile saline. Two hours after this initial injection these animals were injected with 16.7μCi/100 gram body weight of (1-^{14}C)-2-deoxy-D-glucose (53 mCi/mmole) in 0.5 ml sterile saline. Three of the animals were maintained in a quiescent state in a lighted room for a period of one hour. The other three were subjected to horizontal rotary motion at 25 r.p.m. together with a vertical movement of 6 inches at 0.5 Herz for one hour. At the end of this period each animal was anesthetized with ketamine, and the brain was quickly dissected out and frozen in isopentane cooled to -60°C with dry ice. Transverse cryostat sections of the brain stem were cut alternately at 200μm and 20μm from the nucleus gracilis caudally through the superior vestibular nucleus rostrally. Micropunch samples (Eik-Nes and Brizzee, Biochem. et. Biophys. Acta 97, 320, 1965) of the individual vestibular nuclei, the nucleus gracilis, nucleus cuneatus, nucleus of the trigeminospinal tract, nucleus of the tractus solitarius, and the hypoglossal nucleus, at various rostro-caudal levels were obtained from the 200μm sections with a small stainless steel punch measuring 850μm in diameter. The frozen punch samples were prepared for liquid scintillation counting. Differential (^{3}H) and (^{14}C) counts (c.p.m.'s) were made employing external standards. The 20μm sections were prepared for (^{14}C) radioautography by standard methods employing Kodak SB-5 X-ray film.

Data from the scintillation counts revealed that the ^{14}C/^{3}H ratio (c.p.m.'s) was significantly higher ($p = <.03$) in the vestibular nuclei than in the other brain stem nuclei sampled in animals subjected to the motion regimen and higher in the vestibular nuclei in motion-stimulated animals than in those which were not subjected to the motion regimen. The (^{14}C) radioautographs from the motion-stimulated animals revealed a selectively high uptake of 2-DG in the medial and inferior vestibular nuclei as compared with the other brain stem nuclei sampled by the micropunch method. However, heavy grain densities in the motion stimulated monkeys were also observed over the inferior olivary nucleus, lateral reticular nucleus, and the nodulus, uvula and nucleus fastigius of the cerebellum which were not punch-sampled. (Supported by NASA-Ames NAC-2-101 and NIH RR00164)

198.11 CENTRAL NERVOUS SYSTEM GLUCOSE UTILIZATION RATE IN THE NYSTAGMIC RAT. J.W. Patrickson*, F.A. Kutyna, H.J. Bryant, M. Kadekaro, and J.B. Clark* (SPON: G.P. Mueller). Depts. of Physiology and Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD. and Lab Cerebral Metabolism, NIMH, Bethesda, MD.

Methods previously used to identify and analyze central nervous system (CNS) structures involved in the integration of the vestibulo-ocular reflex (VOR) have been limited to a segmented analysis of the system. We used Sokoloff's 2 deoxy-D-[^{14}C] glucose method (J. Neurochem. 28, 897-916, 1977) to comprehensively map the CNS functional activity during activation of the VOR. Male Sprague-Dawley rats (275-300g) were anesthetized with halothane, and the crista ampullaris of the left horizontal canal was surgically destroyed. The lesion resulted in an asymmetric input to the vestibular nuclei which elicted the VOR. After recovery from anesthesia, the freely moving animals developed horizontal nystagmus with the fast component toward the unlesioned (contralateral) side. Glucose utilization rates of corresponding right and left brain structures were compared in sham-operated control animals (N=7) and in lesioned experimental animals (N=7).

Control animals showed no significant differences between corresponding left and right CNS structures. Comparing both sides, glucose utilization rate (mean ± standard error expressed as μm/100g tissue/min) in lesioned animals increased in the following CNS structures: the contralateral medial (122±5††) vs.(90±5) and superior (117±5††) vs. (93±5) vestibular nuclei, the contralateral abducens nucleus (116±6††) vs. (85±4), the ipsilateral interstitial nucleus of Cajal (91±7††) vs. (75±8), and the ipsilateral cerebellar nodulus (128±7††) vs. (86±5). In lesioned versus control animal glucose utilization rate increased in the following structures: the contralateral medial (122±5††) vs. (116±6) and superior (117±5††) vs. (111±7) vestibular nuclei, the ipsilateral interstitial nucleus of Cajal (91±7††) vs. (77±5), and the ipsilateral cerebellar nodulus (128††±7) vs. (98±8). In experimental versus control animals glucose utilization rate decreased in the following CNS structures: the ipsilateral medial (90±5††) vs. (116±7) and superior (93±5†) vs. (111±5) vestibular nuclei, the ipsilateral abducens nucleus (85±4††) vs. (113±7), the contralateral medial rectus motor division of the oculomotor nucleus (85±3†) vs. (95±7), and the bilateral nuclei ambiguii (51±4††) vs. (74±7).

These results provide a quantitative survey of CNS functional activity during nystagmus produced by unilateral horizontal canal ablation.

†Significantly different P<0.01
††Significantly different P<0.001

201 SYMPOSIUM. SUBSTANCE P AS A NEUROTRANSMITTER. I.B. Black, Cornell Univ. Med. Coll. (Chairman); S. Leeman, Univ. Mass. Med. School (Co-Chairman); M. Otsuka*, Tokyo Med. and Dent. Univ.; S. Rosell*, Karolinska Institutet; M.R. Hanley, Imperial College, London; T. Jessell, Harvard Med. School.

Substance P (SP) has been the focus of increasing interest, since accumulating evidence suggests that this undecapeptide may function as a neurotransmitter at a number of loci in the nervous system. M. Otsuka will review relevant studies, and describe electrophysiologic actions of the peptide on peripheral and central neurons. Some of the factors regulating SP expression and metabolism in sympathetic and sensory neurons will be reviewed by I. Black. The pharmacology of the peptide, with particular reference to the evolution of new antagonists, will be discussed by S. Rosell. M. Hanley will describe recent work dealing with the characterization of SP receptors. Finally, T. Jessell will examine the role of SP in pain perception.

202 WORKSHOP. APPLICATION OF VIDEO ENHANCEMENT AND INTENSIFICATION TECHNIQUES TO NEUROBIOLOGY. C. Edwards (Chairman, SUNY Albany), R.D. Allen* (Dartmouth), R.J. Lasek (Case Western Reserve), S.B. Kater (Iowa), R.S. Smith (Alberta), C.A. Mason (N.Y. University).

Allen will explain how AVEC-videomicroscopy produces such a large increase in image contrast that the motion in living cells of "submicroscopic" structures 10-100 nm in diameter may be detected. In squid and lobster nerve the velocities of the bidirectional movements of synaptic vesicles, etc., along microtubules are almost uniform, while larger organelles saltate. Similar events during reticulopodial movement in foraminifers and in cells in culture suggest that "cytoplasmic transport" of vesicles along MTs may be a general process.

Lasek, Brady and Allen have used this technique to visualize particle movements in the squid giant axon. The smallest particles move preferentially in the orthograde direction at rates equivalent to fast axonal transport and appear to correspond to vesicles about the size of synaptic vesicles. Larger particles move in both directions. Remarkably the particle movement continues in axoplasm after removal from the axon by extrusion.

Smith and Kendal have used this technique to investigate the rapid transport of membrane-bound organelles across the Node of Ranvier in frog nerve. Organelle trajectories funnelled towards the node corresponding to the distribution of axonal microtubules. Some organelles hesitated at the entrance to the node and then displayed an axially-oriented oscillation of frequency about 0.1 Hz. Within the node bi-directional organelle transport appeared to be associated with axially arranged structures.

Kater has combined intracellular dye injection with a Silicon Intensified Target (SIT) Camera modified to provide a linear intensity range to view neurons in their normal neuronal and glial surroundings. This alleviates the problems of 1) fluophore fading and 2) photooxidation from high light levels. He has examined the movement of tracers along specific neurite processes as well as the complex morphologies of neurons in invertebrate ganglia, in mammalian brain slices and of growing, regenerating neurites.

Hatten and Mason have studied the migration of developing neurons in culture. It has been proposed that glia are the structural guides for migration of neurons in the developing brain. Video/intensification techniques have been used to study: a) glia process outgrowth; b) association of neurons with glia; c) migration of neurons along glia.

Edwards and Dowd have studied beef chromaffin cells in culture. Normally, the enzyme dopamine-β-hydroxylase (DβH) is present in vesicle membrane and not in plasma membrane. In the presence of Ba^{2+}, which stimulates secretion of chromaffin material, DβH has been found by immunofluorescence techniques and a SIT tube to lie in patches on the cell membrane.

203.1 A MUTATION AFFECTING THE DEVELOPMENT OF AN IDENTIFIED NEURON IN A DROSOPHILA MOTOR SYSTEM. J. B. Thomas, W. J. Costello and D. King (SPON: M. P. Charlton). Biol. Dept., Yale Univ., New Haven, CT 06511, Ohio Univ. Coll. Osteo. Med., Athens, OH 45701 and Southern IL. Univ. Sch. Med., Carbondale, IL 62901.

An X-linked mutation, nj156, has been isolated which affects an identified component of the giant fiber motor pathway in Drosophila melanogaster.

In Drosophila, the giant fiber pathway mediates a visually-induced escape response. In wild-type flies, the giant fiber originates in the brain and descends unbranched via the cervical connective to the thoracic ganglion. Here it forms electrical synapses with the peripherally synapsing interneuron (PSI) and with the motorneuron of the jump muscle, the tergotrochanteral muscle (TTM) (King & Wyman, 1980, J. Neurocytol. 9:753-770; Tanouye & Wyman, 1980, J. Neurophysiol. 44:405-421). The PSI courses dorsally and exits the ganglion in the posterior dorsal mesothoracic nerve (PDMN). Within the nerve the PSI makes chemical synapses onto the five motor axons which drive the wing depressor, the dorsal longitudinal muscle (DLM).

In nj156 adults the giant fiber cannot drive the DLM. The motor axons of this muscle are unaffected; they can be stimulated to produce excitatory junctional potentials in the DLM. This suggests that the normal functioning of the PSI is being affected. EM serial sections through the PDMN reveal that the PSI in nj156 is present, but the axoplasm appears more electron-dense than in normal flies. Pre-synaptic densities are present in the PSI where it contacts the DLM motor axons. However, few or no vesicles are present.

The development of this component was compared in normal and in mutant flies. In normal flies, the PSI axon can be seen in the PDMN by 40 hrs. of pupal development and shows mature synaptic profiles with DLM axons by 70 hrs. In contrast, nj156 flies show no distinct PSI axon within the PDMN at 70 hrs. The PSI axon is either absent at this time or is morphologically abnormal.

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203.2 MONOCLONAL ANTIBODIES TO DEVELOPING DROSOPHILA RETINA. R. M. Lebovitz* and D. F. Ready* (SPON: D. L. Gorlick). Dept. of Bio., Princeton University, Princeton, NJ 08544.

The compound eye of Drosophila melanogaster is composed of over 750 identical ommatidia in a precise hexagonal array. This is established during the third instar larva by a wave of differentiation which sweeps across the eye disc from posterior to anterior. Along a furrow which marks the front of this wave, unpatterned cells are recruited into clusters which are precursors of adult ommatidia. Cells within a cluster appear to be determined to become either photoreceptors or accessory cells at the time of recruitment. To investigate cell surface changes correlated with neural determination, we are selecting monoclonal antibodies to antigens which appear on cells as they acquire their identities at the furrow.

Mice were immunized with membranes isolated from adult fly heads or imaginal discs. The resulting hybridoma lines were screened on eye disc whole mounts using an indirect immunofluorescent assay. Of 255 antibodies screened, three (Drospr 301, 302, and 401) showed a topographic distribution on the disc correlated with pattern formation. We have examined the cellular localization of antigens recognized by the three antibodies. Drospr 302 and 401 binding sites appear on cells immediately behind the furrow, coincident with the first organization of cells into photreceptor clusters. In the light microscope Drospr 302 appears bound uniformly over the surface of photreceptor cells including their axons. Binding sites for 401 are concentrated between the cells in a cluster and may be associated with specialized junctions which hold clusters together. A third antibody, Drospr 301, binds to the apical tips of photoreceptor cells. Unlike Dropsr 302 and 401, Drospr 301 binding sites are first observed on clusters well behind the furrow. A solid-phase radioimmunassay technique show that the sites recognized by these three MAbs are antigenically distinct.

We have examined three eye mutants in which the neural pattern of the eye is disrupted. The first two, rough and lozenge^{50e}, which show a disorganized ommatidial array in the adult exhibit normal binding for all three antibodies. The third, glass^{3} lacks the Drospr 301 antigen although the eye disk appears morphologically normal and binds Drospr 302 and 401 in a wild-type pattern. Adult eyes of glass^{3} mutants do not have rhabdomeres.

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203.3 FIRST DETECTED EYE-OPENING IN A SEGREGATING INBRED STRAIN OF RATS (WLE-c/+): COMPARISON OF GENETICALLY NEARLY-IDENTICAL PIGMENTED VS. ALBINO LITTERMATES. I. S. Westenberg and J. M. Bolam*. Institute for Study of Developmental Disabilit., Chicago, IL 60608.

Previously reported differences between albino rat stocks and pigmented rat stocks in time of eye-opening are possible correlates of albino-pigmented differences in retinal projections. However, the albino stocks differed genetically from the pigmented stocks not only at the albino (c) locus but also at many other loci. Thus, it could not be determined if eye-opening differences were associated specifically with the genetic difference at the c locus. Comparison of albino vs. pigmented mice that differed at the c locus but were otherwise genetically identical showed little or no differences in first eye-opening. This suggested that the previously observed differences in rats were correlates of factors other than just the albino mutation. However, findings in mice may not be generalizable to rats in this case. To test this possibility we used rats in an experiment similar in design to the previous experiments with mice. Our goal was to determine if there is a difference in eye-opening specifically associated with a genetic difference at the c locus in rats.

Litters consisting of at least 1 albino and 1 pigmented rat of the segregating inbred strain WLE-c/+ (Westenberg-Long-Evans) were examined daily for eye-opening. Albino rats had 2 mutant c genes at the c locus (c/c); their black-hooded littermates had 1 mutant c gene and 1 normal gene at the c locus (c/+). In each litter there were 3 possible outcomes: The first eye-opening(s) could be in c/c (a), in c/c and c/+ (tie), or in c/+ (p).

Results in 24 litters were 10 a, 7 tie, and 7 p. The p frequency was larger than expected by chance, but not significantly more so than the a frequency. Thus, in this strain of this species of rodent a difference in first detected eye-opening does not appear to be specifically associated with albinism.

The difference between these results and previous results from rats may be related to the experimental design (within-strain vs. between-strains comparison) or the genotype of the pigmented rats (c/+ vs. +/+). The within-strain design was chosen as more efficient for isolating the effects of a single mutant gene. It is unlikely that c/+ pigmented rats' results would have differed from +/+ pigmented rats', but this remains to be tested. It is possible that c/c-c/+ comparisons in rats of other genetic backgrounds will produce different results.

While differences in first eye-opening of the sort seen in between-strains comparisons were not seen in this within-strain comparison, there is evidence that some aspects of the albino-pigmented retinal projection differences seen in between-strains comparisons are also seen in within-strain comparisons.

Supported by NIH grant EY03013.

203.4 CHARACTERIZATION OF TREMBLER MOUSE SCHWANN CELLS IN VIVO AND IN VITRO. Karl J. Fryxell* and Jeremy P. Brockes (SPON: L.C. Fritz). Division of Biology, California Institute of Technology, Pasadena CA, 91125.

The trembler (Tr/+) mouse mutant has a pronounced reduction in peripheral myelin, while both central myelin and peripheral unmyelinated nerves appear normal. Experiments (by Aguayo, Bunge and collaborators) in which axons of one genotype are confronted by Schwann cells of another genotype (in vivo or in vitro) produce a clear answer---the trembler deficit is expressed in the Schwann cell and not in the axon. The myelin proteins P_0 and P_1 are apparently reduced to the same extent in trembler nerve. We would like to understand the nature of this Schwann cell-specific function, which is required for the accumulation of myelin proteins, but is not required for Schwann cell survival or normal wrapping of unmyelinated axons.

In the original description of the trembler mutation by Falconer in 1951, no difference was found between Tr/+ and Tr/Tr mice in behavior, fertility or lifespan. We have found, however , that homozygotes (of at least one of the Tr alleles) have much less myelin and myelin proteins than heterozygotes do. These homozygotes die at 12-21 days of age, but the juvenile homozygotes may be identified as such by several criteria, including linked genetic markers, behavior, body weight and nerve appearance.

Homozygous trembler Schwann cells show remarkable heterogeneity in their response to competent axons in vivo. Most of these Schwann cells do not make compact myelin, yet they are faintly labeled by a rabbit antiserum to the myelin protein P_0, as detected by indirect immunofluorescence on frozen sections. (Wild-type Schwann cells in unmyelinated nerves are not labeled.) A small fraction of the homozygous trembler Schwann cells, however, are very brightly labeled by the antiserum. Some, perhaps all, of such cells also make compact myelin. Preliminary results suggest that the brightly labeled cells are not distributed along particular "super axons," and also that the occasional production of compact myelin is not correlated with differences in axonal diameter.

We have found that mouse Schwann cells may be immunologically purified by methods similiar to those used for rat Schwann cells (Brockes et al., Brain Res. 165: 105-118, 1979), enabling us to grow "genetically pure" cultures of Tr/Tr Schwann cells. Preliminary experiments indicate that cultured Tr/Tr Schwann cells respond normally to glial growth factor. Additional experiments will be presented to further characterize Tr/Tr Schwann cells in culture.

203.5 SPONTANEOUS OCCURRENCE OF A MUTATION CAUSING SEVERE DEFECTS OF CEREBELLUM AND MOTOR COORDINATION IN MICE. D. Wahlsten, J. Lyons* and W. Zagaja*. Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

In October of 1980 two mice with severe defects of behavioral and cerebellar development were discovered two weeks after birth in a litter of inbred BALB/cCF mice. Breeding of their close relatives revealed that the disorder has a completely recessive mode of inheritance and can be transmitted equally well by males and females. Crosses between known carriers of the defect and crosses between a carrier and the sibling of an affected mouse yielded proportions of affected offspring which were close to the expected Mendelian ratios, indicating single-locus autosomal recessive inheritance.

The syndrome produced by this mutation has several features.

a. At birth about half of the affected mice show a blood bleb under the skull at the anterior margin of the interparietal plate near midline.

b. Within two days of birth, all affected mice have either a short tail or a distinctly blunted tail. Extreme cases have almost no tail at all.

c. All affected mice show delayed development of the righting reflex, and some are never able to right themselves properly. Those which can eventually right themselves and walk exhibit obvious abnormalities of gross motor coordination such as head-tossing, tumbling, chaotic circling and incessant ambulation.

d. The cerebellum is reduced in size and has highly unusual patterns of foliation. In the most severe cases there is only a small amount of cerebellar tissue present at the peduncles. The major cell types in cerebellum appear to be present and in approximately correct positions relative to each other, as seen in Nissl-stained sections.

e. Mortality is very high, and reproduction is impossible. Severely affected animals sometimes die soon after birth or are eaten by the mother. Many others die around the time of weaning, mainly because they are exceedingly active but have difficulty eating solid food.

The syndrome bears a striking resemblance to that produced by the now-extinct "shaker-short" gene (st) in mice reported by Dunn (Proc. Nat. Acad. Sci., 20: 230, 1934). The cerebellar malformation is similar to that found in humans with the Dandy-Walker syndrome, an instance of a Type IV Chiari malformation as discussed by Caviness (Devel. Med. Child Neurol., 18: 103, 1976).

203.6 APPLICATION OF CELLULAR LEVEL HYBRIDIZATION HISTOCHEMISTRY TO THE STUDY OF NEUROENDOCRINE GENE EXPRESSION. C. Gee*, J.L. Roberts* and S.J. Watson (SPON: E. Shapiro). Dept. of Biochem., College of Physicians and Surgeons, Columbia Univ., New York 10032, and Mental Health Resh. Inst., Univ. of Michigan, Ann Arbor, MI 48109.

Progress has been made in the study of gene expression following the advancement of recombinant DNA technology. Routinely, messenger RNA (mRNAs) are extracted from the total tissue and quantitated by specific hybridization to complementary DNA (cDNA) probes. However, due to the low abundance of cells producing specific neuropeptides in neurotissues, and that the same gene can be expressed in various cell types which may be regulated differentially in the same tissue, the study of whole tissue hybridization histochemistry, a technique developed for cellular localization of specific mRNAs can be applied advantageously. It involves the fixation of mRNAs in tissues and hybridization of radiolabeled specific cDNA probes to the section. The cDNA:mRNA hybrids are then autoradiographed and visualized at the light microscope level.

In situ hybridization histochemistry has been refined to the level of single cell resolution in the pituitary. Qualitative information on gene expression at the level of mRNA content can be obtained easily. We have used this technique to study the regulation of propiomelanocortin (POMC) gene expression in the two lobes of the rat pituitary. The absence of glucocorticoids (adrenalectomy) causes an increase in the size and number of POMC expressing cells as well as an increase in autoradiographic grain density, implying an increased POMC mRNA concentration. Treatment of the animal with glucocorticoids gives an opposite effect. Thus, we can distinguish between the regulation of gene expression in normally expressing cells and hormonally induced differentiation of non-expressing cells.

This technique has been used along with classical immunohistochemistry on serial sections to positively identify peptide hormone synthesizing cells. We are working on optimizing the hybridization conditions for the brain. This includes the quantitative fixation of mRNAs, deproteinization to make the mRNA more accessible to probes, and varying the hybridization time, temperature and buffer. ^{32}P-labeled cDNA probes can be used for rapid tissue screening. ^{3}H or ^{35}S can be used for high resolution, but they require longer exposure time. Biotin-derivitized cDNA is being used for both resolution and shorter exposure time. (This work is supported by NIH grant AM 27484 to J.L. Roberts).

203.7 EXPRESSION OF THE PROOPIOMELANOCORTIN GENE IN EARLY STAGES OF FETAL RAT PITUITARY DEVELOPMENT. J. Pintar*, J.L. Roberts*, and C. Gee* (SPON: S. Edelstein). Departments of Anatomy and Medicine, Mt. Sinai School of Medicine, New York, NY 10029. and Dept. of Biochemistry and Center for Reproductive Sciences, Columbia University, New York, NY 10032.

Peptide hormones derived from proopiomelanocortin (POMC) have critical functions during embryogenesis as well as postnatally. Although adult-like anterior and intermediate lobe pituitary POMC processing patterns are established before birth (Pintar, Allen, and Kendall, Endo. Soc. Abst., 1981), little is known about the regulation of POMC synthesis and accumulation in either pituitary or brain during pre-natal development. In order to determine when during development POMC gene expression begins, we have used POMC specific DNA probes (Roberts et al, PNAS 76:2153) to identify POMC mRNA in nucleic acid extracts of different tissues at various stages of fetal development. Pregnant Sprague-Dawley rats were decapitated and fetuses from day 15 and day 19 gestation were dissected. Total nucleic acid was extracted from the pituitary, brain, and liver (whole pituitary from day 15 and separated lobes from day 19) using the proteinase K-SDS method to ensure reproducible mRNA recovery from the small amounts of tissue available. Aliquots of isolated nucleic acid from these tissues were spotted onto nitrocellulose and hybridized with ^{32}P-labelled POMC cDNA. POMC mRNA was detected in pituitary and brain, but not liver, at both stages of development. As in the adult, the pituitary was much richer in POMC mRNA than the brain at these ages. The level of sensitivity was such that the amount of POMC mRNA present in 1/5 total nucleic acid from one embryonic day 15 pituitary was easily detectable. Preliminary results have suggested that POMC mRNA can be detected even at the Rathke's pouch stage, well before POMC has been detected by radioimmunoassay or immunocytochemistry. These results demonstrate that recombinant DNA techniques can detect POMC message levels present in embryonic brain and pituitary glands and suggest that localization of cells synthesizing POMC mRNA by in situ hybridization of POMC cDNA probes should be feasible.

203.8 IDENTIFICATION OF AN ADRENOCORTICOTROPIC HORMONE-LIKE SUBSTANCE IN DROSOPHILA MELANOGASTER BY DNA HYBRIDIZATION AND IMMUNOCYTOCHEMISTRY. C.S. Royden*, P.H. O'Farrell*, E. Herbert*, M. Uhler*, Y.N. Jan and L.Y. Jan. Departments of Physiology and Biochemistry, University of California, San Francisco, CA 94143.

Striking tissue distributions of hormone-like peptides in the nervous system have stimulated intensive efforts to define their function. The power of genetic, cytogenetic and molecular analyses in Drosophila melanogaster could make important contributions to defining the functional significance and genetic complexity of these peptides. Assuming conservation of primary sequence and of function across an enormous evolutionary distance, we have used probes from mammalian systems to detect related peptides immunologically and related coding sequences by DNA hybridization.

We used immunocytochemical techniques to determine whether the Drosophila nervous system contains any peptides homologous to those in the mammalian nervous system. We screened antibodies to 12 mammalian peptides. Three of these, antibodies against ACTH, enkephalin and substance P, specifically stained the Drosophila nervous system and reproductive system. Antibodies to enkephalin and ACTH stained similar structures in the nervous system, including fibers in the optic ganglia, thoracic ganglia and cervical connective. They also produced similar staining patterns in the anterior ejaculation duct of the male reproductive system.

The localization of these immunoreactive materials to neuroactive tissues in organisms as diverse as mouse and Drosophila is a striking result which suggests that these peptides, like acetylcholine, are part of a mechanism so basic to neurologic function that it is conserved across this evolutionary distance. An alternative, and we feel less likely, possibility is that although the sequence is conserved, the function might be unrelated and the localization to nervous tissue is coincidental.

In order to study the structure and function of the ACTH-like substance further, we chose to isolate the gene using DNA hybridization techniques. Using T4 polymerase, we made a ^{32}P-labelled DNA probe from the ACTH portion of pro-opiomelanocortin from mouse cDNA. A bank of Drosophila restriction fragments in the phage λ vector Charon 4 was screened by hybridization with this probe. The DNA was hybridized at three levels of stringency, 15°, 28° and 40° below the melting temperature (Tm) of double stranded DNA. No hybridization to plaques was observed at the highest stringency, while we found several positive plaques at moderate stringency, and many at the lowest stringency. We isolated 10 plaques showing hybridization at moderate stringency; six rehybridized upon rescreening. The DNA from these plaques was isolated, and the amount of homology between these and the original probe is currently under study.

203.9 STUDIES ON THE STRUCTURE OF PRECURSORS TO OXYTOCIN, VASOPRESSIN AND NEUROPHYSINS IN THE RAT USING PROTEIN CHEMICAL AND RECOMBINANT DNA TECHNIQUES. T.G. Sherman*, J.L. Roberts*, J.C. Fiddes* and J.F. McKelvy. (SPQN: Y. Grimm-Jorgensen.) Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794 and Center for Reproductive Science, Columbia Univ., NY, NY 10032 and Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 11724.

Multiple high molecular weight forms of neurophysin (Np) have been translated in vitro using wheat germ extract or rabbit reticulocyte lysate with an RNA fraction enriched in poly(A)- containing RNA species isolated from liquid N_2 frozen rat hypothalamic tissue from normal and 16 day salt-loaded Sprague-Dawley rats, and a homozygous strain of Brattleboro rats. Normal and salt-loaded hypothalamic poly(A) RNA coded for 3 protein species immunoprecipitable with protein A-purified anti-rat Np IgG: 2 proteins of 17,000 daltons with isoelectric points (pI) of 5.28 and 5.60, and a larger, more neutral precursor at 18,500 daltons and of pI 6.15. Homozygous Brattleboro rat poly(A) RNA failed to direct the translation of the 18,500 dalton protein, yet retained the two smaller, more acidic proteins. Using one dimensional reverse phase high performance liquid chromatography, all 3 proteins were shown to contain the 4 cysteine-containing tryptic peptides of mature rat neurophysins. In addition, each protein yielded an additional, unique cysteine-containing peptide.

Given the observed differences in the translatable poly(A) RNAs between Brattleboro and normal rat hypothalamus, and the difficulties in examining subtle differences between the multiple Np precursors by protein chemical methods, we decided to use recombinant DNA methods. Poly(A) RNAs coding for the Np precursors were greatly enriched, with respect to total poly(A) RNA, by fractionation on an isokinetic sucrose-SDS gradient. Aliquots of poly(A) RNA, recovered from each of 25 fractions, were translated in vitro and the distribution of total translation products and immunoprecipitable Np precursors were determined along the gradient. Fractions of RNA displaying the greatest Np precursor enrichment (approx. 20-fold) served as templates for oligo(dT)-primed cDNA synthesis. These single stranded ^{32}P-labeled cDNAs were used as probes to screen a rat hypothalamic cDNA library containing approximately 6000 recombinants. Nearly 60 positive clones were identified, several of which generated restriction fragments of predicted size, based on restriction sites reflecting known amino acid sequences of rat neurophysins. Further screening and sequencing of recombinants so identified are in progress and should provide definitive information on the relationship between the multiple forms of Np precursors.

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203.10 CLONING OF COMPLEMENTARY DNA TO PHENYLETHANOLAMINE-N-METHYLTRANSFERASE. E.E. Baetge, H.M. Moon*, B.B. Kaplan, D.H. Park, D.J. Reis and T.H. Joh. Lab of Neurobiology and Dept. of Anatomy, Cornell Univ. Med. Coll., New York, NY 10021 and Lab of Molecular Neurobiology, N.Y.S. Inst. for Basic Research, Staten Island, NY 10314.

In order to study the expression, organization and structural relationship of the genes involved in catecholamine biosynthesis, we sought to synthesize and clone complementary DNA (cDNA) to poly(A)mRNA coding for the catecholamine synthesizing enzymes. We report here the cloning of cDNA to phenylethanolamine N-methyltransferase (PNMT) which catalyzes the conversion of norephinephrine to epinephrine.

Specific PNMT poly(A)mRNA was isolated from bovine adrenal medulla by affinity chromtography of PNMT antibody-polysome complexes on a Protein A Sepharose column. Polyribosomal mRNA bound to the column was eluted with 20 mM EDTA in 25mM Tris-Cl, pH 7.6, and poly(A)mRNA was purified by oligo-dt cellulose chromatography. With this procedure, PNMT mRNA was enriched 50-fold as judged by translation and quantitative immunoprecipitation of a 31,000 MW protein with PNMT antibody. PNMT mRNA thus enriched was used as a template for the synthesis of cDNA. The 3' OH terminus of double stranded cDNA was extended with 10-15 deoxycytosine residues and inserted into the Pst 1 site of poly dG tailed plasmid PBR322 (15-25 residues per 3' OH terminus). The resultant recombinant plasmids were used to transform competent E. coli strain 294. Tetracycline-resistant, ampicillin sensitive clones were screened by positive hybridization selection, and preliminary analysis identified 2 clones containing PNMT cDNA inserts, pPNMT-22 and pPNMT-24. pPNMT-22 cDNA insert was isolated from the plasmid DNA by digestion with Pst 1 and found to be approximately 350 base-pairs in length. The pPNMT-22 cDNA insert was labeled by nick translation with ^{32}P-dCTP to 1 x 10^8 cpm/ug and used in Northern blot analysis. The pPNMT-22 probe strongly hybridized to an RNA species of approximately 1000 nucleotides, a size appropriate for a mRNA coding for a 31,000 MW protein

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203.11 CONSTRUCTION OF A RECOMBINANT DNA LIBRARY CONTAINING SEQUENCES COMPLEMENTARY TO HUMAN ADULT CORTEX POLYADENYLATED RNAs. A. J. Michael*, R. B. Gammon*, S. Pardue*, D. E. Croall*, D. Sparkman*, W.S.T. Griffin, and M. R. Morrison. Departments of Neurology and Cell Biology, University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235.

Construction of a recombinant cDNA library containing sequences encoded by adult human brain is a prerequisite for the study of transcriptional and post-transcriptional changes during normal and abnormal human brain development. Undegraded polyadenylated RNAs, representative of those present in vivo, were isolated from adult human cortex (Morrison, M. and Griffin, S., Anal. Biochem., 113: 318, 1981). This RNA was used as a template for the synthesis of a recombinant DNA library first strand cDNAs were reverse transcribed from the polyadenylated RNAs with 20-40% efficiency, and denaturing gel electrophoresis demonstrated that full length cDNAs had been synthesized. The single-stranded cDNAs were tailed at their 3' ends with approximately 30 dC residues. Double-stranded cDNAs were then synthesized, using oligo (dG) as a primer for the reverse transcriptase. The double stranded cDNAs were cloned into the Pst I site of plasmid pBR322, using the standard (dG), (dC) tailing procedures (Villa-Komaroff, L. et al., PNAS, USA, 1975: 3727, 1978). Transformation efficiencies of E coliK12 RR1 were 100-250 tetracycline-resistant, ampicillin-sensitive clones/ng double-stranded cDNA. Thus, one microgram of tailed, double-stranded cDNA yielded a library of at least 100,000 clones. The library was stored on Millipore filters as 1) a semi-ordered collection, 2) isolated DNA, and 3) glycerol stocks of transformed cells. Insert sizes were mostly between 600 and 1100 base pairs.

Clones will now be isolated which correspond to the different mRNAs encoding the α_1, α_2, β_1, and β_2 tubulin subunits so that the structure of these mRNAs can be compared. Clones corresponding to developmentally-regulated mRNAs will also be identified and used to study the regulation of their expression. Supported in part by NIH 14886 and grants from the Leland Fikes and the Chilton Foundations.

203.12 REGULATION OF TYROSINE HYDROXYLASE mRNA ACTIVITY IN A RAT PHEOCHROMOCYTOMA AND ISOLATION OF A cDNA CLONE FOR TYROSINE HYDROXYLASE. E.J. Lewis*†, A.W. Tank*§, N. Weiner§ & D.M. Chikaraishi*† (SPON: J. Masserano). †Dept. of Molec. & Cell. Biology, National Jewish Hospital/National Asthma Center; and §Dept. of Pharmacology, U. of Colorado Health Sciences Center, Denver, CO 80206.

A number of variant cell lines which respond differentially to glucocorticoid and cyclic AMP derivatives with respect to induction of tyrosine hydroxylase (TH) were isolated from the rat pheochromocytoma PC12 cell line. Cell cultures, maintained in RPMI supplemented with 10% horse and 5% fetal calf sera, were incubated for 3 days with either dexamethasone (dex) (1 μM) or 8BrcAMP (1mM). RNA was extracted from cells and mRNA activity for TH (mRNA-TH) was determined following translation of total RNA in an in vitro protein synthesizing system derived from rabbit reticulocytes, using ^{3}H-leucine as tracer. Total translation products were then immunoprecipitated with antisera raised to purified tyrosine hydroxylase and the amount of radioactivity in TH was compared to that of total translation products. In a representative experiment, the mRNA-TH activity in one of these cell lines was elevated from a basal level of 0.067% of total translational activity to 0.2% in cultures incubated with 8BrcAMP and to 0.5% in cultures incubated with dex. The mRNA-TH activity in cultures containing both inducers was 0.85% of total translational activity.

A cDNA clone to mRNA-TH was isolated from a cDNA library constructed from polyA+-mRNA highly enriched in mRNA-TH. Double-stranded cDNA was made using AMV reverse transcriptase and E. coli DNA polymerase. This cDNA was inserted into the Pst I site of plasmid pBR322 using the G-C tailing technique, and the recombinant plasmids used to transform the E. coli strain HB101. Clones containing inserted sequences were screened by hybridization of plasmid DNA to ^{32}P-cDNA made from RNA isolated from induced and non-induced cell cultures. Those clones demonstrating differential hybridization between these two probes were further screened using hybrid selected translation and immunoprecipitation. From this screening, a clone was identified which contains approximately 350 base pairs which are complementary to mRNA-TH.

204.1 SEROTONIN AND NOREPINEPHRINE INNERVATION PATTERNS IN VISUAL CORTEX OF THE RAT: REGIONAL SPECIALIZATION. M.E. Molliver, M.S. Lewis*, R. Grzanna, and H.G.W. Lidov. Dept. Cell Biology/ Anatomy, Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

The patterns of serotonin (5-HT) and norepinephrine (NE) innervation of cerebral neocortex in the albino rat have been studied with particular attention to the laminar distribution of axons in visual cortex, employing antibodies directed at 5-HT and DBH.

Overall, the 5-HT innervation density is relatively low in dorsal neocortex and is greater in more ventro-lateral areas. There is a corresponding change in axon morphology and distribution. The 5-HT axons have small varicosities with smooth, elongated intervaricose segments of varying length. DBH-positive axons have far more numerous varicosities. In area 17, the density of 5-HT axons is highest in layer I and slightly less in V and VI. There is a somewhat lower density in layers II-III and a markedly low density in layer IV. Many 5-HT axons in layers I, V, and VI are tangentially oriented in both sagittal and coronal planes, whereas in layers II-III, a radial orientation is common, best seen in parasagittal sections.

The 5-HT axon density in area 17 is far less than in SI, the most outstanding difference being the decreased density in layer IV of visual cortex. In contrast to the 5-HT distribution, NE axons in visual cortex have a higher density of fine arborizations in layer IV, and the axons are more evenly distributed across the layers.

The 5-HT axons exhibit a high degree of regional specialization most evident in visual cortex where the pattern of innervation differs from 5-HT in other areas (e.g., SI), and from that of NE axons. The main new finding is that the 5-HT innervation in rat visual cortex is strikingly different from that in primate area 17 where the 5-HT axons are especially numerous in layers IVA and IVC but very low in V and VI (cf. Morrison et al., PNAS 79:2401-2405, 1982).

The 5-HT axons are likely to contact different classes of cortical neurons in different species as well as in different cortical areas. The 5-HT raphe-cortical innervation pattern is not diffuse but exhibits regional specificity and order. Consequently, the functional role of 5-HT axons is different in separate cortical areas and differs from that of the NE projections. Moreover, the functions of the monoamine projections in primate visual cortex are likely to differ from those in the rat. Studies are in progress to determine whether a particular subset of MA neurons projects to visual cortex. [Support: NIH Grants #NS-15199 and NS-08153]

204.2 PHYSIOLOGICAL AND MORPHOLOGICAL ANALYSIS OF AFFERENT AXONS IN THALAMO-RECIPIENT LAMINAE OF MACAQUE STRIATE CORTEX. G.G. Blasdel and J.S. Lund. Depts. of Anatomy and Ophthalmology, Medical University of South Carolina, Charleston, S.C. 29425.

Morphological analysis of afferent axons entering macaque striate cortex, following intracellular recording and HRP filling, reveals consistent patterns of arborization. Six axons judged physiologically to derive from magnocellular LGN laminae (because they lacked color selectivity, and responded transiently with high contrast sensitivity to flashed stimuli) were observed to terminate primarily in layer 4c-alpha with sparse projections into 6. All spread in a stripe-like or patchy fashion and cover large areas when viewed normal to the pia. Reconstruction of axons against ocular dominance bands, following eye injection with H-3 Proline, reveals that the patchy or stripe-like terminations fall largely within one eye's set of ocular dominance 'columns'.

Three axons with sustained, color-opponent receptive fields (characteristic of parvocellular LGN laminae) were found to terminate either in layer 4c-beta alone or in layers 1 and 6 (1 axon). The two axons arborizing in 4c-beta conform to a highly stereotyped pattern also observed in many other 4c-beta axons, recovered following white matter injections of HRP. Their preterminal trunks follow long tortuous trajectories through the grey matter before terminating in highly circumscribed clumps that fill the thickness of 4c-beta but extend no farther than 200 micra in any lateral direction. The tangential area covered by a single 4c-beta axon is approximately 1/6 that observed for a single 4c-alpha axon but the density of boutons per unit volume is about the same. A different pattern is seen for the axon projecting into layers 1 and 6; this axon, which had a blue-ON center, arborizes in 4 separate patches and spreads over a distance greater than 2 mm. Axons with small terminal fields in layer 4a, recovered following white matter injections of HRP, have a honeycomb appearance when reconstructed in 3 dimensions and viewed normal to the pia. These axons can also contribute sparse projections to laminae 2-3.

Physiological recordings with metal electrodes confirm earlier observations (Hubel, Wiesel and LeVay, 1974) of a precise retinotopic map in both 4c-alpha and 4c-beta. In addition, they reveal a difference in contrast sensitivity which is greater for non-oriented units in 4c-alpha than it is for non-oriented units in 4c-beta. Given the enormous spread of afferent axons in 4c-alpha (up to 1.2 mm in some cases), it is unlikely that the observed retinotopic map, which can be seen within the width of a single ocular dominance column, derives directly from the geometry of afferent inputs.

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204.3 HISTOCHEMICAL SUBDIVISIONS OF THE MACAQUE'S LATERAL GENICULATE BODY AND STRIATE CORTEX: DEMONSTRATION BY PSEUDOCHOLINESTERASE AND ACETYLCHOLINESTERASE STAINING AND EFFECTS OF EYE ENUCLEATION. A.M. Graybiel and C.W. Ragsdale, Jr.* (SPON: R. Held). Dept. of Psychology and Brain Sci.,Mass. Inst. of Tech.,Cambridge,MA 02139

We have compared the distributions of butyrylcholinesterase (BuChE), acetylcholinesterase (AChE) and cytochrome oxidase in sets of serial frozen 30-50μm sections through the lateral geniculate body (LGN) and visual cortex of 9 adult Rhesus macaque monkeys. Five of the monkeys were normal, 4 had undergone unilateral (left) eye enucleation 5-30 days before perfusion.

In the LGN, the most striking finding was that BuChE staining was intense in the parvocellular layers but very weak in the magnocellular layers. Eye enucleation resulted in a sharp and progressive decline in BuChE staining in the denervated parvocellular layers. By contrast, all LGN cell-layers were stained with AChE and eye enucleation did not change the AChE staining patterns noticably. Cytochrome oxidase was also present in all cell-layers but declined after denervation.

In area 17, BuChE and AChE were both distributed in highly differentiated layering patterns which distinguished striate from peristriate cortex. BuChE was densest in layers 4Cα and upper 4Cβ and at the borders of layer 5. The base of layer 4 was pale. In the infragranular layers there were hints of periodicity and BuChE-positive cells. AChE staining was densest in layers 1, 4A, 4C and 6. In addition, both enzymes were distributed in a gridwork of 100-300μm-wide patches visible in tangential sections through the supragranular layers. These patches were much more prominent in BuChE than in AChE and sometimes precisely coincided, but sometimes were clearly out of phase, with cytochrome oxidase patches visible in adjoining sections.

Monocular eye enucleation produced, at all survival times, regularly alternating 300-500μm-wide stripes of high and low cytochrome oxidase activity in tangential sections through layer 4 as described previously for 10 day enucleates by Horton and Hubel (1981). A striking stripe pattern also appeared in AChE sections through layer 4 where, most clearly in the 10 day animal, 300-500 μm-wide AChE-rich and AChE-poor bands alternated. In BuChE sections stripes of high enzyme activity were also present, but they were thin and difficult to visualize except in tangential sections through the layer 4/5 border. The stripes of high AChE and BuChE activity were in register with one another but, remarkably, corresponded to the stripes of low cytochrome oxidase activity.

We conclude that both pseudocholinesterase and acetylcholinesterase stains mark functional subdivisions of the macaque primary visual pathway and may prove valuable and at least partly independent markers in studying the responses of this system to altered visual input. Supported by NIH 5R01 EY02866 and 5P30 EY02621.

204.4 ORIENTATION SHIFT BETWEEN UPPER AND LOWER LAYERS IN MONKEY VISUAL CORTEX. B.M. Dow, R. Bauer*, A.Z. Snyder*, R. Vautin*. Neurobiology Division, School of Medicine, SUNY, Buffalo, NY 14226.

A major feature of Hubel and Wiesel's orientation column model (J. Comp. Neurol. 158:267-294, 1974) is constant orientation preference of all cells in a line running perpendicular to the surface from layer 2 to layer 6. Our systematic experiments using vertical or nearly vertical penetrations (Exp. Brain Res. 41:54-60, 1980) have led us to question the validity of this feature. In particular, we have found a major shift in preferred orientation of recorded cells as our electrode crosses over from the supragranular to infragranular layers. This finding has now been documented histologically with measurements of penetration angles and recording sites.

In a series of 57 penetrations in foveal striate cortex of awake monkeys trained to fixate according to the paradigm of Wurtz, we have recorded single cells or cell clusters at regular intervals of 200-400μ from pial surface to white matter, and have used a computer-assisted method to measure orientation of the preferred stimulus at each recording site. The data include 379 recording sites, an average of 6 sites per penetration. In the great majority of penetrations orientation as a function of depth in the supragranular layers could be reasonably well approximated by a straight line. The mean slope of this line for 57 penetrations was 20.7°/mm. In 12 marked penetrations the mean penetration angle with respect to the surface normal was 17.7°.

As the electrode entered the lower layers there was typically a shift in orientation preference. Subsequent lower layer cells tended to have nearly the same orientation as the first (shifted) cell. On the basis of 7 penetrations with well localized electrolytic lesions the shift site was determined to be at the border between layers 4C and 5. The size of the orientation shift between upper and lower layers was determined quantitatively by extrapolating separately obtained least squares fitted lines for upper and lower layer orientation (as a function of depth) to the midpoint between the cell pair exhibiting the orientation shift. Among 57 penetrations 40 (70%) fell into a large-shift group with shift sizes ranging from 45-90° (mean 70.2°), and 17 (30%) fell into a small-shift group with shift sizes ranging from 0-44° (mean 18.7°).

The data thus indicate a major shift in orientation between the supragranular and infragranular layers, and are suggestive of intracolumnar inhibition, presumably mediated by inhibitory neurons projecting either down from upper layers or up from lower layers.

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204.5 LATTICE-LIKE INTRINSIC NEURAL CONNECTIONS IN PRIMATE STRIATE VISUAL CORTEX. K.S. Rockland and J.S. Lund. Dept. of Ophthalmology, Medical Univ. of S.C., Charleston, S.C. 29425.

Horseradish peroxidase (HRP) injections were made in striate cortex of squirrel and macaque monkeys. These injections (0.5-1.2 mm in diameter) resulted in periodic patches of HRP label, transported in both the orthograde and retrograde direction, around the injection site. In squirrel monkeys, the HRP-labeled intrinsic connections occur at two cortical levels (layers 2-3 and 4B) and form a lattice-like pattern near the injection site. The walls of the lattice preferentially include labeled cell bodies and axon terminals, with no labeled neurons in the regularly spaced lacunae within the lattice walls. Axon segments course in the walls and also cross through the central lacuna region. Further from the injection, the pattern in layers 2-3 consists of more punctuate HRP labeled loci with interconnecting axon trunks. This shift in pattern presumably reflects lower levels of HRP accumulation. A similar pattern is seen in the macaque in layers 2-3, although the exact periodicity is not identical in the two species: the punctuate HRP loci are spaced about 500-600 μ apart in macaque and about 350-450 μ apart in squirrel monkeys. In both species, the supragranular layers are known also to contain an ordered array of cytochrome oxidase rich loci. Alternate sections were stained for cytochrome oxidase and HRP activity (40 μ tangential sections matched by blood vessels). In both primates the cytochrome rich loci do not regularly coincide with the punctuate HRP pattern. In squirrel monkey the cytochrome dots (450-500 μ apart) lie within the walls of the more densely labeled HRP lattice, and both the HRP and cytochrome systems surround the same regularly arranged series of lacunae. This suggests that the HRP labeled system constitutes a fixed-position network. In squirrel monkey, similarly structured but more extensive connectional lattice is labeled by HRP in layer 4B (where no cytochrome rich loci occur). The 4B lattice includes both pyramidal and spiny stellate neurons (only pyramidal neurons are stained in the supragranular lattice), and has been mapped over 2-3mm from the injection site (vs. 1.5mm for layers 2-3). The two patterns appear in register, but do not show marked interconnections, with a characteristic unlabeled gap between them in lower 3, 4A. A connectional lattice in 4B is also indicated in macaque. The supragranular HRP lattice resembles the pattern and scale of deoxyglucose (DG) uptake in visual cortex after stimulation with lines of single orientation (Horton and Hubel, '81). The various patterns of DG uptake (eg; lines vs. lattice) after different stimuli may be correlated with specific but different subsets of the anatomical lattice which are emphasized under different conditions of visual stimulation. (Supported by EY03321).

204.6 CLUSTERED INTRACORTICAL CONNECTIONS IN CAT VISUAL CORTEX. C.D. Gilbert and T.N. Wiesel. Dept. of Neurobiology, Harvard Medical School, 25 Shattuck St., Boston, Mass. 02115.

Lesion studies have shown that intrinsic cortical connections (those which are restricted to a given area) can extend for several millimeters parallel to the cortical surface (Fisken et al, 1975; Creutzfedlt et al, 1977). From both intracellular (Gilbert and Wiesel, 1979) and extracellular (Gilbert and Wiesel, 1981; Rockland and Lund, 1982) tracing studies, it has been demonstrated that axons participating in these connections do not give off collaterals all along their length, but instead tend to give them off in clusters which are separated by gaps with relatively little innervation.

In the present work we attempted to examine these connections in greater detail. Two days following a focal extracellular injection of horseradish peroxidase (HRP) into a particular cortical area, the label was distributed in clusters of cells surrounded by diffuse granular labeling. These clusters were found at considerable distances from the injection site. More clusters, covering a larger proportion of the area, were observed after injections in area 18 than after injections in area 17. It was not possible to determine the degree to which this labeling pattern reflected diffusion and differential uptake versus axonal transport from the injection site.

From the intracellular HRP injections in area 17 we observed a number of pyramidal cells in superficial and deep layers showing clustering of their axon collaterals. When reconstructed using a 3-dimensional computer graphics system and rotated to obtain a view tangential to the cortical surface, these axons exhibited numerous clusters and were seen to extend over considerable distances (3 to 6 mm). Cells in layer 2+3 projected both to distant points within their own layer as well as within layer 5. The axon of such one cell had clusters covering the same area in both layers, and the clusters in layer 5 were located directly under those in layer 2+3. Surprisingly, some pyramidal cells did not project into the white matter, and formed intrinsic connections exclusively. Finally, the axonal fields of all our injected cells have been asymmetric, extending for greater distances along one cortical axis than along the orthogonal axis. This may have implications concerning the functional role of these connections.
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204.7 LIGHT AND EM ANALYSIS OF CYTOCHROME OXIDASE-RICH ZONES IN THE STRIATE CORTEX OF SQUIRREL MONKEYS. E. Carroll* and M. Wong-Riley (SPON: F.D. Anderson). Dept. of Anat., Med. Coll. of Wis., Milwaukee, WI 53226.

Oxidative metabolism in the central nervous system has been demonstrated by cytochrome oxidase (C.O.) histochemistry (Wong-Riley, '79). In the primate striate cortex, distinct patterns of high C.O. activity were found in laminae II, III, IVA, IVC and VI (Horton & Hubel, '80; Humphrey & Hendrickson, '80). We sought to extend these studies at both the light and EM levels to determine the cellular and subcellular distribution of reactivity within the striate cortex of squirrel monkeys. Cells with high C.O. staining were observed throughout the grey matter and included mostly stellate (lam 2-6) and some pyramidal (lam 2-3 and 5-6) neurons. Intermittent puffs of C.O. activity were observed in lam 2-3 as described before; their average diameter was 210μm and were spaced about 500μm apart (center-to-center). Puffed areas alternated with areas of less intense C.O. activity whose dimensions were roughly 250μm. Approximately 2/3 of the cells located within the puffed areas were intensely stained whereas in adjacent inter-puffed areas only 1/4 of the cells were considered reactive. Electron microscopic examination of puffed areas revealed that reactive neurons of the stellate variety usually contained 2-3 times as many mitochondria as did non-reactive cells; their nuclei were often greatly invaginated and their cytoplasm stained more intensely. An occasional reactive pyramidal cell was also observed. Non-reactive cells were smaller with scanty cytoplasm and had few if any symmetrical axo-somatic synapses. Asymmetrical axosomatic synapses were observed thus far only on moderately and highly reactive cells. In non-reactive interpuffed areas, fewer cells were intensely reactive. Dendritic profiles contained 30-40% of the total population of mitochondria within the puffs. Of these, 1/2 were very reactive and 1/3 moderately reactive. Approximately 30% of the total population of mitochondria resided in axon terminals. Of these, 50% were moderately reactive and were found equally in terminals forming symmetrical synapses with flattened vesicles or asymmetrical synapses with round vesicles. 20-30% of axonal mitochondria were intensely reactive and were more prevalent in terminals of the symmetrical type. 30-50% of the mitochondria in myelinated axons were reactive.

These results indicate that cytochrome oxidase activity in reactive puffed areas of the striate cortex is localized within certain neuronal types and primarily within dendritic profiles. Reactive puffed areas also contained at least twice as many metabolically highly active neurons as did adjacent non-puffed regions.

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204.8 CYTOCHROME OXIDASE BLOBS IN MONKEY AREA 17: RESPONSE PROPERTIES AND AFFERENT CONNECTIONS. David H. Hubel and Margaret S. Livingstone, Department of Animal Neurobiology, Harvard Medical School, Boston, Massachusetts 02115

Despite forces to the contrary we have managed to extend our studies of cytochrome oxidase blobs in striate cortex of macaque and squirrel monkeys. As reported last year, cells in cytochrome oxidase rich regions of layers II and III (blobs) show poor orientation tuning or none at all. In the macaque the blobs lie in rows centered in the ocular dominance columns.

Not surprisingly, in the macaque, cells in the blobs were strongly monocular, much more so than cells outside the blobs. Cells in any one blob always favored the same eye. In the squirrel monkey, which has poorly defined ocular dominance columns, the blob cells were more binocular.

Some of the blob cells strongly resembled cells in the ventral layers of the lateral geniculate (Wiesel and Hubel, 1966); in particular many had broad band spectral sensitivities, with centers and surrounds differing in their sensitivities to red, and highly sustained responses evoked from the very large receptive field surrounds. Firing tended to occur in rhythmic bursts. Other blob cells were more like dorsal layer cells in showing clear opponent-color responses. We have seen both Type-II cells (no receptive field surround, color-opponent centers) and double-opponent cells (e.g. center: on to long wave lengths, off to short; surround: off to long, on to short). We saw one extraordinary binocular cell, clearly a single unit, with double-opponent properties in each eye but of opposite sign.

After proline injection into the lateral geniculate body, puffs of label were seen in layers II and III, and these puffs coincided with the blobs. This suggests that the blobs have different inputs from non-blob regions but leaves open the question of whether the input is direct from the geniculate or transneuronal via layer IV. After injection into the lateral geniculate of horseradish peroxidase (not wheat-germ conjugated) the blobs were likewise labeled. We understand that ordinary horseradish peroxidase, unless intracellularly injected, is only rarely transported transneuronally (Mesulam, 1982), and therefore suspect that the blobs receive direct geniculate input. Since layers IVA and IVC also stain intensely for cytochrome oxidase it seems that darker cytochrome oxidase staining may correspond to regions with geniculate afferents. Consistent with this is the fact that lesions in the lateral geniculate produce in the cortex patches of pale staining in layers IVA and C and in the blobs.

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204.9 THE ORGANIZATION OF CORTICAL MODULES IN PRIMATE STRIATE CORTEX, Roger B. H. Tootell* Martin S. Silverman* Eugene Switkes* and Russell L. De Valois* Dept. Psychology, Univ. Calif. Berkeley, Berkeley, Calif. 94720. (spon: G. H. Jacobs).

The arrangement of orientation and ocular dominance columnar systems into discrete cortical modules has been suggested previously (Hubel and Wiesel, 1977), but evidence for such modules has been sparse. We here present positive 2DG evidence for cortical modules and describe aspects of their internal structure.

We have examined (in the same flat-mounted sections) the location of cytochrome oxidase (CO) spots and the pattern of 14C-2-Deoxyglucose (2DG) uptake produced by various visual parameters. In each visual condition, the topographic relationship between the CO and 2DG patterns in layer III was quantitatively analyzed by computer. The systematic relationship of 2DG patterns (produced by various types of stimuli) to the CO spots allowed us to develop a model of the cortical module. We find that CO spots lie in the center of ocular dominance strips, and at the borders of orientation columns. All our 2DG evidence is consistent with a lack of orientation specificity in the CO spots, relative to interspot regions. We also see clear evidence for a columnar organization related to spatial frequency. In animals which were binocularly shown a high spatial frequency grating at all orientations, 2DG uptake was maximal around the CO spots and minimal on the spots. The converse 2DG pattern was seen in animals which were binocularly shown a low spatial frequency pattern at all orientations. Since these 2DG patterns extended through all cortical layers except layer IVc, this constitutes preliminary evidence for spatial frequency columns in the monkey. Further, orientation columns produced by high spatial frequency stimuli may differ from those produced by multi-frequency stimuli. One animal was shown a horizontally-oriented high spatial frequency grating: this produced a pattern of 2DG strips (except in layer IVc) which run exactly perpendicular to the ocular dominance strips in the same tissue. Another animal shown a multi-spatial frequency pattern at the same orientation showed no such unusual 2DG pattern.

We see, then, a clear mapping of function relative to the CO spots. Suprisingly, we also see an obvious structural correlate of the CO spots. We have found conspicuous spot-like increases in myelination in striate layer III, with the same periodicity as the CO spots. Less apparent myelin-heavy spots can also be seen in layers V and VI. We also see myelin-heavy strips running through the middle layers of V2: these are almost certainly related to the CO strips which we have reported to run through V2 (Tootell and Silverman, Neurosci. Abstr. '81).

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204.10 SPATIAL INVARIANCE OF RECEPTIVE FIELD LOCATION IN THE PRESENCE OF EYE MOVEMENTS OF FIXATION FOR NEURONS IN MONKEY STRIATE CORTEX. B. C. Motter* and G. F. Poggio. Dept of Physiology, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205

It is usually assumed that in visual cortex the location in space of a neuron's receptive field has a fixed topographic correspondence to a specific location on the retina. Thus, an accurate definition of the location and spatial organization of cortical receptive fields should take into account the precise position of the eye in relation to the position of the stimulus in the visual field. We have examined the effect of changes in eye position during fixation on the response of neurons in the striate cortex to a moving bar stimulus. The onset time of the response was taken as an indicator of the position of the response field of the neuron. All neurons examined were located within the central 3 degrees of visual field representation. The positions of left and right eyes were measured by an infrared corneal reflex oculometer with a resolution of 2 min-arc. All aspects of stimulus and behavioral control and data collection were under computer control. In our experimental conditions, rhesus monkeys trained to fixate without eye position feedback align their visual axes on the target with a monocular spatial variability of about 8 min-arc (1 SD) in both horizontal and vertical directions. Accordingly, the position of the response field of a cell during fixation should have a minimum variability of about 8 min-arc. Stimuli sweeping through a receptive field at a rate of 2 degrees/sec should therefore evoke responses with an onset variability of about 70 msec (1 SD). Our measurements on a set of cells driven by such stimuli and with a well defined response onset yielded an average variability of 30 msec. Moreover, by directly correlating eye position with response onset we observed that during fixation the cortical neural response to a sweeping stimulus is independent of the actual position of the eye along the axis of stimulus motion. The timing of the neural discharge accurately signals the position of the stimulus relative to the fixation target and does not reflect small changes in eye position. Indeed, changes in the position of the fixation target, or of the stimulus, by as little as 6 min-arc are consistently reflected in changes in the time of occurrence of the neural discharge indicating that neuronal sensitivity for the position of visual objects depends also on a mechanism for the spatial invariance of receptive field position during fixation. These observations suggest that the location of the receptive field of striate neurons remains dynamically stable, representing a region of space which is not displaced to and fro by the small eye movements of fixation.

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204.11 ORGANIZATION OF MOVEMENT AND DIRECTION SENSITIVITY IN AREAS 17 AND 18 OF CAT CEREBRAL CORTEX. B. R. Payne and N. Berman. Depts. of Anatomy and Physiology/Biochemistry. The Medical College of Pennsylvania, Philadelphia, PA 19129.

Direction of movement is an important parameter for neurons in areas 17 and 18 of cat cerebral cortex but, unlike cells sensitive to a particular axis of movement (orientation selectivity), the functional architecture of direction sensitive neurons is not clear. An understanding of the organization of direction sensitive neurons in areas 17 and 18 is important because these two regions are responsible for the direction sensitivity of neurons in other cortical and subcortical visual centers. In areas 17 and 18, the magnitude of the difference in preferred direction between two nearby neurons may be either small (close to 0°) or large (close to 180°). Small differences reflect changes in axis-specificity while large differences reflect reversals in preferred direction. In a previous study, we have shown that these two types of changes are organized systematically in the laminar dimension and result in a grouping of neurons preferring similar directions. To investigate the possibility of a radial organization to this grouping we made a series of electrode tracks perpendicular to the cortical surface. Along each track a series of closely spaced isolated neurons was recorded. Electronically controlled stimuli were presented and quantitative methods of data collection were used to determine the range of movement directions of a bar stimulus over which each neuron responded; its preferred axis of movement and its response to movement in the two possible directions along this axis. We found, in agreement with others, that axis of movement is highly organized in the radial dimension. However, the direction sensitivity of neurons along this dimension shows a different but still highly organized pattern. Neurons more sensitive to one of the two possible directions of movement along a movement axis tend to be segregated together in the upper cortical layers. The neurons more sensitive to movement in the opposite direction tend to be located deeper in the same cortical column. This grouping of neurons was occasionally interrupted by cells showing axis specificity with little or no direction sensitivity. These non-directional cells were usually recorded in the middle cortical layers. Therefore, the common direction preferences of cells extends across some but not all cortical laminae, i.e. neurons in the supragranular layers are more sensitive to one direction of movement while neurons in the infragranular layers are more sensitive to the opposite direction. This organization may be related to the rich reciprocal connections between the supragranular and infragranular layers within one cortical column.

204.12 MULTI-MICROELECTRODE RECORDING FROM THE STRIATE CORTEX OF THE CAT, AIMED AT ESTABLISHING SYNCHRONY BETWEEN NEURONS. C. R. Legendy* (SPON: H. G. Vaughan, Jr.). Dept of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Extracellular single-unit recordings were made from the striate cortex of the unanesthetized cat, scanning natural objects in the room. Temporal linkage between spikes of the recorded units was sought by means of a digital computer. Instead of gathering the spikes into a cross correlogram as is done more often in correlation studies, the computer was programmed to detect temporally confined events termed spot correlations. These are epochs characterized by nearly periodic firing by two or more units, lasting usually 2-4 such periods, with all participating units having nearly the same period. Spot correlations are interpreted as manifestations of synchronized ignitions extending over larger neuron pools. Statistical significance is computed from the variance of the nearly-equal interspike intervals and interchannel latencies, and from the rate of pattern recurrences. The latter are defined as temporally close occurrences of patterns having nearly the same period and interchannel latency; e.g., several repetitions in a few minutes.

205.1 REDUCTION OF NEURONAL DEATH BY EMBRYONIC NEUROMUSCULAR BLOCKADE PERSISTS AFTER HATCHING. R. W. Oppenheim. Neuroembryology Lab, Dorothea Dix Hospital, Raleigh NC 27611

Chronic treatment of chick embryos with neuromuscular blocking agents during the period of naturally-occurring death of spinal motoneurons [embryonic (E) day 5 to E10] has previously been shown to result in a virtually total reduction of neuronal death. Embryos retain these "extra" motoneurons as long as the neuromuscular blockade is maintained; once normal embryonic neuromuscular function resumes, however, a delayed cell death occurs. It was of considerable interest to know whether there is a critical period after which neuromuscular blockade is no longer necessary for maintaining the "extra" motoneurons. Because chronic neuromuscular blockade results in impaired pulmonary respiration, treated embryos typically die after hatching (E21).

Recently, however, 4 curare-treated embryos survived hatching and were kept alive for 3-4 days posthatching. Despite being unable to stand and locomote normally (owing to severe ankylosis and muscle atrophy), these chicks were active and moved about by lying on their sides and making vigorous wing flaps and alternating leg movements. This indicates that any residual curare that was present at hatching was rather quickly excreted or metabolized. On the 3rd or 4th day, posthatching, animals were deeply anesthetized and sacrificed by cardiac perfusion with Carnoys fixative. Brachial and lumbar spinal cords with adjacent sensory and sympathetic ganglia were removed, processed for paraffin histology, sectioned (15 μm) and stained with thionin. Select limb muscles were processed for acetylcholinesterase (AChE) histochemistry. Motor, sensory and sympathetic neurons were counted; additional morphometric measurements were also carried out. At present only the lumbar spinal cord has been evaluated. The curare-treated animals had 20,250 ± 1,803 motoneurons in the lateral motor column (LMC) versus 9,660 ± 1,057 for the controls ($p<0.001$). By contrast, there were no differences between curare and control animals in the following measures: number of sensory or sympathetic ganglion neurons; size of LMC, sensory or sympathetic neurons; number of nucleoli in LMC, sensory or sympathetic neurons; length or volume of the lumbar spinal cord. Muscles (AChE) and brachial spinal cords are presently being examined.

It will be necessary to examine animals that survive for considerably longer following chronic neuromuscular blockade in ovo, before one can conclude that the reduction of cell death is permanent. Nonetheless, the present results indicate that 3-4 days of posthatching neuromuscular activity does not result in the loss of the "extra" motoneurons. This differs from the situation in ovo and therefore suggests that these neurons may have passed a critical period after which neuromuscular blockade is no longer necessary for their maintenance.

205.2 ACTIVITY PATTERNS OF CHICK HINDLIMB MUSCLES WITH INAPPROPRIATE INNERVATION. M.J. O'Donovan* and Lynn T. Landmesser. Dept. of Biology, Yale University, New Haven, CT 06511.

We have previously reported that hindlimb flexor and extensor muscles in stage 34-36 chick embryos are activated in an alternating manner during spontaneous or evoked movement sequences in an isolated spinal cord-hindlimb preparation (O'Donovan et al., Neurosci. Abstr. 7:688, 1981). Using this preparation we have now examined the activation patterns of 35 inappropriately innervated hindlimb muscles in 23 embryos, in which one limb had either been shifted anteriorly (3-4 segments) or rotated about the A-P or D-V axis. Surgery was performed at stage 16-18 which is prior to motoneuron outgrowth into the limb bud. In 12 embryos the motoneurons innervating one of the experimental muscles were retrogradely labeled with horseradish peroxidase (HRP), which enabled their identity to be determined from the position of their cell bodies within the spinal cord (Landmesser, L. T., J. Physiol. 284:371, 1978).

Muscle activity was recorded electromyographically during spontaneous or evoked movement sequences in stage 34-38 embryos. In seven cases the recorded activity pattern was opposite (flexor vs extensor) to that normally found for that muscle. In the majority of cases (28) however the abnormally innervated muscles were activated in a functionally appropriate manner.

For 12 muscles (in 12 embryos) the identity of their motoneurons was established using retrograde HRP labeling. In each case the motoneurons were activated according to their original identity rather than the normal function of the muscle they synapsed with.

We conclude that the central connections responsible for generating these movements are not influenced by the motoneuron's peripheral target. The fact that the majority of abnormally innervated muscles behave appropriately appears to reflect a preferential innervation by functionally appropriate motoneurons. This may occur because the rules governing the peripheral projection patterns within the limb may restrict a motoneuron's choice of peripheral target (Lance-Jones, C. and Landmesser, L.T., Proc. R. Soc. Lond. B. 214:19, 1981).

205.3 LOCALIZATION OF CHOLINESTERASE PATCHES IN PARALYZED AND UNINNERVATED SKELETAL MUSCLE DURING IN VIVO DEVELOPMENT. G. S. Sohal, Dept. of Anatomy, Medical College of Georgia, Augusta, GA 30912.

Acetylcholinesterase (AchE) is primarily associated with the basal lamina at the vertebrate neuromuscular junction. The mechanism by which AchE becomes localized in the synaptic cleft during the course of embryonic development is little understood. In vitro studies on rat or chick myotubes suggest that the appearance of AchE patches is dependent on the presence of neurally-evoked muscle contractile activity. However, studies on Xenopus myotubes suggest that accumulation of AchE on muscle membrane may not require neural influences. Because of the conflicting views on the regulation of AchE in birds and mammals on one hand and amphibians on the other hand this problem was reexamined during in vivo development. Duck embryos were paralyzed either presynaptically with botulinum toxin or postsynaptically with curare. Fifteen μ/g botulinum toxin or 2 mg curare in 0.1 cc saline solution was dropped onto the vascularized chorioallantoic membrane twice daily from day 9 onward. Daily motility observations indicated that the embryos were completely paralyzed. In some embryos the superior oblique muscle was made aneural (uninnervated) by permanently destroying the trochlear motor neurons in the midbrain with an electrocautery on embryonic day 7; three days prior to the arrival of motor nerve fibers in the muscle. Embryos were sacrificed on day 17 and the superior oblique muscles were fixed in 4% phosphate-buffered formaldehyde and processed for cholinesterase staining according to the method of Karnovsky. In both paralyzed and aneural muscles patches of AchE were observed on the muscle membrane. In the paralyzed muscle the reaction product was often associated with a region of nerve-muscle contact showing postsynaptic infoldings and densities. In the aneural muscle reaction product was sometimes associated with muscle membrane without any infoldings and at other instances surface invaginations were present. Sometimes AchE patches appeared to coexist with basal-lamina like material. AchE staining was absent when muscles were preincubated with neostigmine or when eserine sulfate was added to the incubation media. These observations suggest that accumulation of AchE in the developing duck muscle is independent of neural influences.
(Supported by grants from NIH and MDA).

205.4 PHYSIOLOGICAL AND NEUROANATOMICAL CHANGES FOLLOWING CHRONIC NEURAL BLOCKADE IN XENOPUS EMBRYOS. Lanny J. Haverkamp. Neurobiology Progr., Univ. N. Carolina, Chapel Hill, NC 27514 and Neuroembryology Lab., Dorthea Dix Hosp., Raleigh, NC 27611.

In an extended and quantified replication of the classic studies of Harrison, Carmichael, et al., we have earlier demonstrated the behavioral effects of drug-induced (chloretone, lidocaine, or α-BTX) immobility on the development of swimming behavior in Xenopus embryos (Haverkamp and Oppenheim.1981. Soc. Neurosci.Abstr.7:181.). After approx. 30 hrs. of drug exposure (during that developmental period when motility normally appears and is refined), experimental animals exhibited quantitative, though not qualitative, abnormalities in swimming behavior when compared to stage-matched control embryos. After this period, which extends from 2-48 hours after removal from the drug (depending upon the drug used), experimental and control animals were behaviorally indistinquishable.

The physiological activity of primary motoneurons was studied in normal and in experimental embryos subjected to the same drug treatment paradigm used in the behavioral studies. Recordings were made from suction electrodes placed bilaterally between the myotomes. Mean values and variability of phase coupling of activity on the two sides as well as burst duration as a percent of period were similar amoung groups at each stage studied. Period length was significantly greater in recovering experimental animals during those stages at which they demonstrate quantitative behavioral deficiencies.

The neuroanatomical effects of neural blockade were studied through comparisons of the lengths and arborizations of primary motoneuron dendrites, retrogradely filled with HRP. Quantifications of dendritic length, thickness, branching, and Scholl analyses indicate that early embryonic exposure to either chloretone or lidocaine results in a distinct decrease in dendritic numbers, length and branching. It appears at this time that the neuroanatomical differences between normal and experimental animals gradually subside after their removal from the drug environment, though definitive statements cannot yet be made due to the wide variability of the increasingly complex dendritic arborizations of single neurons.

Drug-induced blockade of functional activity during a period encompassing the onset of embryonic motility results, therefore, in only transient alterations of motor system development. Although the bases for these disturbances are not yet clear, these studies indicate that early alteration of function does not result in any permanent effects on motor system structure or function.

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205.5 NEURONAL PLASTICITY: ITS RELATION TO ELECTROPHORETIC MIGRATION IN RESPONSE TO POSTSYNAPTIC POTENTIAL GRADIENTS. Barry Horwitz. Physics Department, Texas Woman's University, Denton, Texas 76204.

A postsynaptic mechanism for transforming electrical activity at a synapse into structural modifications near the synapse is proposed. The firing of a synapse produces a postsynaptic potential (PSP) which is attenuated as it spreads electrotonically into other parts of the neuron. The potential difference so generated gives rise to an intraneuronal electric field, and permits the electrophoretic migration of charged metabolites, both along the inner surface of the fluid membrane and in the cytoplasm. It was demonstrated by Poo (Ann. Rev. Biophys. Bioeng. 10: 245-276, 1981) that, when placed in an external electric field, both concanavalin A and acetylcholine receptors within approximately 15 minutes accumulate toward the cathodal site of cultured myotubes, 30 μm in diameter. The field strengths used were 1-10 V/cm. We will show that the internal electric fields generated by PSPs can be of comparable magnitude. Therefore, we suggest that these fields can play a significant role in locally organizing the distribution of charged species, thus allowing differential biochemical activity at different synaptic locations. Three diverse sets of experimental observations are used to illustrate the electrophoretic effect. They are (1) the relationship between the number of preganglionic axons innervating ciliary ganglion neurons, and the number of major dendrites possessed by the ganglion neurons (Purves and Hume, J. Neurosci. 1: 441-452, 1981); calcium action potentials in growth cones (Grinvald and Farber, Science 212: 1164-1167, 1981); and, membrane heterogeneity in myelinated axons (e.g., Waxman, Trends in Neurosci. 4: 7-9, 1981).

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205.6 APPORTIONMENT OF AXON TERMINALS TO TARGET NEURONS IN A MAMMALIAN AUTONOMIC GANGLION. Richard I. Hume and Dale Purves. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

The apparently competitive process by which patterns of innervation are established during development is not well understood. A major obstacle has been the difficulty of assessing the way in which individual axons branch within a target structure and apportion their terminals on the surfaces of particular neurons. We report here a study of these aspects of innervation in a simple neuronal system, the ciliary ganglion of adult rabbits.

Electrical recording and intracellular staining of individual preganglionic axons with horseradish peroxidase (HRP) showed that each axon innervates only about 10-20 of the approximately 400 ganglion cells. In whole mounts, neurons whose cell bodies were enveloped by HRP-labeled boutons from a single axon were surrounded by other somata which appeared to receive no contacts from the labeled axon. Electron microscopical examination of labeled presynaptic terminals on individual ganglion cells confirmed that the boutons of single axons were sharply confined to particular target neurons. Labeled terminals on individual postsynaptic profiles (either cell bodies or dendrites) were not usually mixed with unlabeled endings. Thus the terminals of a labeled axon appeared to be relatively segregated from the terminals of other axons on the surface of individual postsynaptic cells.

Component steps in the synaptic responses of multiply innervated ganglion cells were usually smaller than synaptic potentials in singly innervated neurons; thus an axon probably makes fewer boutons on each of the neurons it shares than on neurons that it captures completely. Moreover, neurons innervated by several different axons tended to have fewer synapses on their somata than neurons innervated by only one or two preganglionic axons.

Taken together, these findings suggest that the correlation between the dendritic geometry of ganglion cells and the number of different axons that innervate them (Purves & Hume, 1981) arises from a reduction in the competition between axons innervating the same cell, perhaps as a result of terminal segregation on dendritic arbors.

References

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205.7 NEURONAL GEOMETRY, REFLEX FUNCTION, AND ACTIVITY PATTERNS OF RABBIT CILIARY GANGLION CELLS. David A. Johnson and Dale Purves. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

Evaluation of the suggestion that dendritic geometry modulates competition between axons innervating the same target cell (see Hume and Purves, 1981 and preceding abstract) requires considerable additional information about the neurons involved. In the work reported here we asked how the geometry of individual ciliary ganglion cells is related to their reflex function and to neuronal activity.

Of three hundred ganglion cells impaled with microelectrodes in anesthetized rabbits, nearly all (293) showed tonic synaptic input from preganglionic axons, even in darkness. Two hundred and eighty-one of the neurons studied showed a change in this synaptic drive upon retinal illumination. The majority of ganglion cells (86%) increased their rate of discharge in response to illumination of the ipsilateral retina; many cells, however, were inhibited by retinal illumination (41%), usually of the contralateral retina. Some of the cells that were inhibited by illumination of one eye were also excited by illumination of the other eye.

Fifty neurons whose reflex behavior to light had been characterized were subsequently stained with horseradish peroxidase. There was no obvious correlation between the reflex response of cells to retinal illumination and their dendritic geometry. Thus neurons with similar reflex properties included cells lacking dendrites altogether as well as cells with complex dendritic arborizations; conversely, neurons with similar geometries often had different reflex characteristics.

With respect to temporal patterns of synaptic activity, the frequency of tonic (and reflex) activity tended to increase in proportion to the the degree of multiple innervation. However, different inputs to a multiply innervated cell fired without apparent relationship to one another.

These findings show that the range of dendritic geometry and number of inputs previously observed in this ganglion (Purves and Hume, 1981) is not incidental to the presence of neurons with different reflex behaviors in the ganglion. Neither can the persistent multiple innervation of some ganglion cells be explained by synchronous activity of their several inputs. On the other hand, these observations are consistent with the idea that dendrites regulate convergence by modulating competition between axons innervating the same neuron.

References

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205.8 OPTIC NERVE SECTION DELAYS SYNAPTIC RE-ARRANGEMENT IN NEONATAL RABBIT CILIARY GANGLIA, Patrick C. Jackson.† Dept. Physiol. & Biophys., Washington Univ., St. Louis, Mo 63110.

During the first weeks of normal post-natal development there is a marked reduction, without cell death, in the number of preganglionic axons innervating individual rabbit ciliary ganglion cells (Johnson & Purves, 1981). Thus, at birth virtually all cells are innervated by more than one axon (on average ≈5/cell) whereas by four weeks of age 25-35% of the cells are innervated by only one axon.

Light entering the eye after about 10 days of age (just prior to eye opening) normally causes a reflexive contraction of the ipsilateral iris sphincter which receives motor innervation from ciliary ganglion cells. To test whether neuronal activity plays a role in the elimination of preganglionic inputs, ganglionic activity was altered by severing one or both optic nerves intracranially in newborn rabbits; since the rabbit shows little consensual light response, unilateral optic nerve section should reduce substantially light-induced neuronal activity in the ipsilateral ganglion.

The number of preganglionic axons innervating individual ciliary neurons was determined in vitro by counting the increments in the recorded response of ganglion cells impaled with microelectrodes 2-32 wks after optic nerve section. Cutting one optic nerve at birth delayed, but did not prevent, the usual reduction of polyneuronal innervation in the ipsilateral ciliary ganglion; no such delay was evident in the contralateral ganglion. Animals subjected to bilateral nerve section showed a delay in the elimination of preganglionic inputs similar to that produced by unilateral nerve section.

Age	Number of Inputs (Mean ± SEM) n > 100 cells/group		
	Normal	Optic Nerve Cut	
		Contralateral	Ipsilateral
birth	4.6 ± 0.1		
2 weeks	3.3 ± 0.1	3.3 ± 0.1	3.8 ± 0.1
4 weeks	2.3 ± 0.1	2.3 ± 0.1	3.4 ± 0.1
8 weeks	2.4 ± 0.1	2.5 ± 0.1	2.5 ± 0.1

Evidently the time course, but not the final outcome, of input elimination is influenced by the level of activity through this developing reflex circuit.

Johnson, D.A. & Purves, D. (1981). J. Physiol. 318: 143-160.

†Multiple Sclerosis (Canada) Postdoctoral Fellow.

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205.9 MEMBRANE POTENTIAL AND THE REGULATION OF ACETYLCHOLINE RECEPTOR SYNTHESIS IN EMBRYONIC CHICK MUSCLE CELLS. B. H. Shieh*, L. Pezzementi and J. Schmidt. Dept. of Biochemistry, State Univ. at N.Y. at Stony Brook, Stony Brook, N. Y. 11794.

The effect of elevated extracellular potassium on acetylcholine receptor synthesis was studied in chick embryonic muscle cultures. Receptor synthesis was measured by assaying appearance of -bungarotoxin binding activity over a 24-hr period. At physiological ionic strength, potassium chloride, in the 3.3 mM to 50mM range, gave rise to a complex dose-response curve whose prominent features are a considerable reduction of receptor appearance rate at 20 mM and a more than 2 fold increase at higher concentrations. The effect of potassium chloride on receptor synthesis appeared to be fairly specific: neither was there a duplication of its effect by other electrolytes or solutes, nor did it alter total protein synthesis or receptor stability by more than 30% at any concentration tested; cellular acetylcholinesterase levels actually declined with increasing KCl concentrations. In order to explore the mechanism of the potassium effect, tetrodotoxin (10^{-6}M), veratridine (3 X 10^{-6}M), D-600 (1.6 X 10^{-5}M), and ryanodine (3 X 10^{-7}M) were tested in the presence of various concentrations of potassium. Sodium channel toxins as well as calcium effectors modified the potassium response. Based on these findings we propose that the effects of potassium are due to (a) cessation of spontaneous muscle activity upon raising KCl from 3 to 10 mM; (b) depolarization of the muscle membrane and persistent activation of a calcium channel as concentration is raised from 10 mM to 20 mM; (c) finally, inactivation or desensitization of the calcium channel, or some other signaling element proximal to the sarcoplasmic reticulum, upon further depolarization.

205.10 DISTRIBUTION OF ACETYLCHOLINE RECEPTORS AND SYNAPTIC BASAL LAMINA PROTEOGLYCAN IN NORMAL AND DENERVATED XENOPUS SARTORIUS MUSCLE. Diana Card Linden and M. J. Anderson*. UCLA School of Medicine, Department of Physiology, Los Angeles, CA 90024 and Carnegie Institution of Washington, Baltimore, MD 21210.

Xenopus sartorius muscles were reacted with fluorescein labeled monoclonal antibodies specific for synaptic basal lamina proteoglycan (SBL-antibody) and tetramethylrhodamine labeled α-bungarotoxin (R-αBGT). In all normal and contralateral control muscles dense localized accumulations of basal lamina proteoglycan were coincident with clusters of junctional acetylcholine (ACh) receptors, revealed by the R-αBGT staining. These proteoglycan accumulations commonly extended laterally beyond the subneural aggregates of junctional ACh receptors and sometimes were observed extending beyond the distal extent of organized ACh receptor staining in tips of terminal branches. In occasional examples, an entire terminal branch stained with SBL-antibody, but was devoid of ACh receptor clusters. Extrajunctional SBL-antibody staining was diffuse and of lower intensity than that at the neuromuscular junction, and frequently appeared to be organized into fine radially distributed fibrils.

After 3-4 weeks of denervation many endplates showed normal patterns of both SBL-antibody and R-αBGT staining. However, in some cases, diffuse perijunctional ACh receptor clusters were observed and these were commonly associated with similar regions of increased SBL-antibody staining. After 5 weeks denervation, dense ACh receptor accumulations were present in endplate-free regions of muscle fibers, and these also were usually associated with plaques of increased SBL-antibody staining. By this time most junctional ACh receptor clusters were notably disorganized, with extensive diffuse perijunctional ACh receptor staining, associated in most cases with corresponding accumulations of SBL-antibody. However, within individual junctions some well-defined bands of normal SBL-antibody and ACh receptor stain still remained.

On the basis of these observations we conclude that the organization of ACh receptor and basal lamina proteoglycan are under coordinate regulation by the motor neuron in adult muscle fibers. This research was funded by an NRSA Award #NS-07101 to DCL.

205.11 NORMAL SENSITIVITY TO ACh OF INACTIVE BUT INNERVATED MUSCLES. E. J. Muñoz-Martínez, L. Colín-Barenque* and J. Cueva*. Depto. Fisiología y Biofísica, CINVESTAV, Apartado Postal 14-740, 07000 México, D.F. MEXICO.

The present experiments were designed in an attempt to clarify whether or not the ACh-supersensitivity of denervated muscle fibres results from the lack of muscle activity.

Sensitivity to ACh was studied in the denervated as well as in the innervated but inactive rat soleus muscle. Motor inactivation was produced by spinal cord section; unilateral section of the sciatic nerve was also performed in the spinalized rats. The inactivity of the innervated muscle was assesed by chronic electromyography.

Three to five days after the surgical procedures both the denervated and the innervated soleus muscles of spinalized rats showed comparable atrophy. The denervated soleus showed dose-dependant ACh contractures, extrajunctional depolarizations to iontophoretically applied ACh and an increase in the binding of ^{3}H-α-bungarotoxin. The contralateral, inactive but innervated soleus showed no ACh contractures and the ACh sensitivity was restricted to the end-plate. A small increase in toxin binding per mg of dry tissue was detected in these muscles but there was no increase when the binding was corrected for muscle atrophy.

To exclude the possibility that small levels of undetected muscle activity could have prevented the development of ACh-supersensitivity in the innervated soleus of spinal rats, some denervated muscles were chronically stimulated at 0.05/sec for 4 days. The increase in toxin binding induced by denervation was not changed by the stimulation even though the muscle atrophy was less pronounced than in the contralateral, innervated soleus, suggesting that the denervated and stimulated soleus was more active than the inactive, innervated ones.

It is concluded that the lack of muscle activity does not play a significant role in the induction of denervation supersensitivity.

205.12 ABRUPT SUPPRESSION OF ACETYLCHOLINESTERASE PRODUCTION IN DENERVATED RAT DIAPHRAGM. S. Brimijoin, J. A. Edwards. Department of Pharmacology, Mayo Clinic, Rochester, Minn. 55905.

Previous work in this laboratory has shown that the 4S (monomeric) form of acetylcholinesterase (AChE) has a much faster apparent turnover than do the other forms of this enzyme in rat diaphragm (Brimijoin and Carter, J. Neurochem. 38: 588, 1982). 4S AChE is also the first form to decrease in activity when diaphragm is denervated (Carter and Brimijoin, J. Neurochem. 36:1018, 1981). We now report evidence that the production of AChE monomer by rat diaphragm is drastically curtailed within 1 hr of denervation.

The left hemidiaphragms of 8 Sprague-Dawley rats (200-250g) were denervated by intrathoracic transection of the phrenic nerve (stump length, 5 mm). After 8 hr the animals were exsanguinated by perfusion with 0.9% NaCl and the muscles of these and of 5 unoperated control rats were removed and homogenized (in 50 mM Tris HCl, pH 7.4, 1M NaCl, 1% Triton X-100, 0.2 mM EDTA). Samples were spun at 10,000 g for 10 min. The supernatants were then fractionated on 5 ml linear sucrose density gradients (5-20%) by ultracentrifugation (120,000 g for 16 hr).

As determined by a radiometric assay for enzyme activity, roughly equivalent amounts of the 3 major forms of AChE were resolved (ie, 4S, 10S, and 16S enzyme). Of the forms, 10S AChE showed the same activity in denervated and control diaphragms (2.2 ± 0.1 and 2.2 ± 0.3 units, respectively); 16S AChE activity rose nonsignificantly after denervation (to 2.0 ± 0.1 from 1.5 ± 0.2 units); and 4S AChE activity fell by 40% (to 1.2 ± 0.1 from 2.0 ± 0.1 units, $p < 0.001$). Exactly the same amount of 4S AChE activity was lost in 5 left hemidiaphragms incubated in vitro in oxygenated physiological salt solution for the same duration (final value, 1.2 ± 0.1 units).

To test whether the loss of monomeric AChE after denervation reflected reduced production or increased degradation of enzyme, animals were treated with the protein synthesis inhibitor, cycloheximide (20 mg/kg, s.c., every 4 hr). In innervated diaphragms the activity of 4S AChE fell to 0.8 units after 8 hr of treatment with cycloheximide. In diaphragms denervated at the onset of treatment the activity fell to 1.2 ± 0.1 units. This result implies that the halflife of monomer was even longer in the denervated muscle (10.8 hr) than in the innervated muscle (6 hr). Therefore, loss of monomer after denervation stems from reduction in synthesis only. With an 11 hr halflife it would take a full 8 hr for denervated muscles to lose 0.8 units of monomer, even with zero synthesis. Therefore the effect of denervation on the production of 4S AChE must begin almost at once.

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206.1 IMPACT OF FOOD DEPRIVATION ON HYPOTHALAMIC α-ADRENERGIC RECEPTOR ACTIVITY AND NOREPINEPHRINE (NE) TURNOVER IN RAT BRAIN. M. Jhanwar-Uniyal*, F. Fleischer*, B.E. Levin and S.F. Leibowitz (SPON: N.E. Miller). Rockefeller Univ., New York, NY 10021 and Dept. Neurosci., N.J. Medical School, Newark, NJ 07103.

Catecholamine (CA) agonists alter feeding behavior when injected directly into specific hypothalamic areas. The medial paraventricular nucleus (PVN) has been identified as the most responsive brain area to NE, which stimulates food intake through α-adrenergic receptors. In contrast, the lateral perifornical area (PFH) is particularly responsive to dopamine and epinephrine, which suppress food intake through dopaminergic and β-adrenergic receptors. The present study examined the impact of food deprivation and subsequent feeding on α-adrenergic receptor binding in 15 discrete hypothalamic and extra-hypothalamic areas. The impact of deprivation on NE turnover was also examined.

Male albino rats, on a 12:12 light-dark cycle, were exposed to 5 different time intervals of food deprivation and sacrificed (at 5:00 A.M.) one hr prior to onset of light. The rats were deprived of food (water was provided ad lib) for either 0 hrs (N=5), 6 hrs (N=3), 12 hrs (N=3), 24 hrs (N=3), or 48 hrs (N=5), with an additional group (N=4) deprived for 42 hrs and then permitted to eat for the next 6 hrs. The rats were sacrificed by decapitation, and discrete brain areas were microdissected. Standard radioligand binding procedures were employed, with the antagonist ^{3}[H]WB-4101 (1.0 nM) used to label α-adrenergic receptors. The microenzymatic assay (Levin et al., 1980) was used to measure NE turnover in 4 brain areas of 7 satiated and 7 48-hr food-deprived rats injected with α-methyl-p-tyrosine.

The results demonstrate that: 1) of 15 brain areas examined, only two areas, namely the PVN and PFH, exhibit any change in α-adrenergic receptor binding in response to food deprivation. 2) The PVN showed a large decrease in specific ^{3}[H]WB-4101 binding after 12, 24 and 48 hrs deprivation. (For example, after 48 hrs binding in satiated rats, 314.3 fmoles/mg protein, decreased by 84% to 49.6 fmoles/mg protein ($p<.001$) in deprived rats.) 3) The PFH showed a large increase in specific ^{3}[H]WB-4101 binding after 12, 24 and 48 hrs deprivation. (After deprivation, binding increased from 123.1 fmoles/mg protein in satiated rats to 363.8 fmoles/mg protein ($p<.01$) in deprived rats.) 4) After 48 hrs deprivation, a significant increase ($p<.025$) in NE turnover was observed in the PVN, with no change occurring in the PFH, ventromedial and dorsomedial nuclei. 5) In rats permitted to eat for 6 hrs after 42 hrs of deprivation, the down-regulation of PVN α-adrenergic receptors was significantly attenuated. These results indicate that food deprivation produces dramatic and site-specific effects on α-adrenergic receptors and NE turnover in the hypothalamus. (This study was supported by MH 22879.)

206.2 EFFECT OF CENTRALLY ADMINISTERED NEUROTENSIN ON MULTIPLE FEEDING PARADIGMS. A.S. Levine, J. Kneip*, M. Grace* and J.E. Morley. Neuroendocrine Research Laboratory, VA Medical Center, Minneapolis, Minnesota, 55417 and the Departments of Food Science and Nutrition and Medicine, University of Minnesota, St. Paul-Minneapolis, Minnesota.

The tridecapeptide, neurotensin, produces a variety of effects when administered into the central nervous system, including a decrease in body temperature, locomotor activity and pain thresholds. Recently, it has been reported that intraperitoneal administration of neurotensin suppressed food intake in 3 hr food deprived rats. We have previously postulated that dopamine and the endogenous opiates act as a tonic signal for the induction of feeding, being held in check by monoamines and neuropeptides (Life Sci. 27:355, 1980). The present study was undertaken to examine the effect of neurotensin on multiple paradigms known to stimulate feeding. Following a 30 hour starvation period, neurotensin suppressed feeding at the 20 µg (1.5±0.2 g/30 min, $p<0.01$), 10 µg (1.6±0.6 g/30 min, $p<0.25$) but not at the 1 µg (1.8±0.6 g/30 min) dose when compared with saline controls (2.6±0.3 g/30 min). Norepinephrine (20 µg ICV) induced feeding (1.7±0.2 g/30 min) was suppressed at the 20 µg neurotensin dose (0.9±0.2 g/30 min, $p<0.01$) but not at the 10 µg (1.2±0.3 g/30 min), or 1 µg (1.3±0.2 g/30 min) dose. In contrast, neurotensin did not suppress muscimol (the gamma amino butyric acid agonist) (500 ng ICV) induced feeding (2.9±0.5 g/30 min) at the 20 µg (2.0±0.5 g/30 min), 10 µg (2.0±0.4 g/30 min) or the 1 µg (3.2±0.7 g/30 min) dose. Insulin induced feeding (10 units) (3.6±0.3 g/3 hrs) also was not suppressed by neurotensin at the 20 µg (4.3±0.5 g/3 hrs), 10 µg (2.6 ±0.5 g/3 hrs), or 1 µg dose (3.6±0.4 g/3 hrs). Dynorphin, the putative endogenous opiate kappa agonist, induced feeding (1.8± 0.3 g/hr) when administered ICV (10 µg). Neurotensin suppressed dynorphin induced feeding at the 20 µg (0.9±0.2 g/hr, $p<0.05$) and 10 µg (0.9±0.2 g/hr, $p<0.05$), but not at the 1 µg dose (1.3±0.4 g/hr). Recently it has been shown that several anorectic peptides, including CCK and somatostatin, suppress feeding via the vagus. In the present study, subdiaphragmatic vagotomized rats were given ICV neurotensin (20 µg) or vehicle at 2000 hrs and food intake was measured for the ensuing 2 hours. Neurotensin suppressed spontaneous feeding ($p<0.01$) in vagotomized rats (2.5±0.3 g/2 hrs) when compared with saline controls (4.2±0.5 g/2 hrs) suggesting that an intact vagus is not necessary for neurotensin's anorectic effect. Thus, neurotensin suppressed feeding initiated by food deprivation, norepinephrine and dynorphin but not that initiated by muscimol or insulin. We conclude that neurotensin may play a role in short-term appetite regulation by a complex interaction with monoamines and neuropeptides, particularly norepinephrine and the kappa opiate agonist, dynorphin.

206.3 CONTINUOUS AND PHASIC INFUSION OF NOREPINEPHRINE (NE) INTO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) INCREASES DAILY FOOD INTAKE AND BODY WEIGHT IN RAT. Sarah F. Leibowitz, Constantin Marinescu* and Saul S. Lichtenstein*, Rockefeller Univ., NY 10021.

Previous studies have demonstrated that a single manual injection of NE directly into the PVN will elicit a feeding response in satiated rats. Multiple manual injections of NE throughout the day (4 injections over 7 daytime hours) are effective in potentiating eating at each time of injection and consequently increasing total daily food intake (Roossin, Rosenn and Leibowitz, 1980). The present study examined the impact on ad lib feeding of chronic remote infusions (continuous and phasic) of NE into the PVN.

Male albino rats (350-400g) were chronically implanted with a Khavari cannula-swivel assembly aimed at the PVN. They were tested in an open-top chamber, which permitted their cannula-swivel to be connected to the injector needle and polyethylene tubing arrangement attached to a Hamilton gas-tight microsyringe (see Myers, 1977). After a few days of adaptation to the testing procedure, the experiment, which lasted 20 days, was initiated. The protocol consisted of two 10-day sequences, during which time the rats were infused, in counterbalanced order, with either 0.04% ascorbic acid in saline or NE dissolved in ascorbic acid (12 nmoles/1.0 µl/hr). For some rats (N=6), this infusion, of NE or vehicle, was given continuously throughout the 10-day sequences, whereas for other rats (N=6), the infusion was phasic, with a 0.5 µl injection occurring at 30-min intervals. The rats were maintained on an ad lib feeding schedule, and food intake (lab chow powder + milk) and body weight measurements were taken daily.

The results indicate that chronic noradrenergic stimulation of the PVN is effective in modulating total daily food intake and body weight. With the ascorbic acid infusion, the rats' daily food intake was 29.1±3.6g with continuous infusion and 32.8±2.4g with phasic infusion. With infusion of NE, daily food intake was increased by 11.3% ($p<.05$) with continuous infusion and by 14.9% ($p<.01$) with phasic infusion. This potentiation of eating was observed consistently throughout the entire 10-day sequence. Body weight was similarly increased by NE. Under ascorbic acid conditions, the rats' baseline rate of weight gain ranged from 1.2 to 1.6g/day. This increased, by approximately 100% ($p<.05$), to 2.9g/day with continuous NE infusion and to 3.2g/day with phasic NE infusion. Over the course of the 10-day NE sequence, the rats gained approximately 10% of their original body weight, compared with 3-5% under ascorbic acid conditions.

These results, along with preliminary data obtained with chronic PVN infusion of a catecholamine synthesis inhibitor, indicate that the α-noradrenergic system of the PVN is effective in altering long-term feeding patterns and body weight gain. (This research was supported by grant MH 22879.)

206.4 EVIDENCE THAT THE KAPPA OPIATE RECEPTOR IS INVOLVED IN THE INITIATION OF FEEDING. J.E. Morley, A.S. Levine, M. Grace* and J. Kneip*. Neuroendocrine Research Laboratory, Minneapolis VA Medical Center, Minneapolis, Minnesota, 55417 and the Departments of Medicine and Food Science and Nutrition, University of Minnesota, Minneapolis-St. Paul, Minnesota.

A large body of evidence has suggested a role for the endogenous opiates and their receptors in the regulation of appetite. Recently we have shown that dynorphin, the putative kappa agonist, produces feeding in rats (Life Sci. 18:1901, 1981). In this study we have examined the relative effects of ketocyclazocine (KC), cyclazocine, and ethylketocyclazocine, all kappa agonists, butorphanol, a kappa-sigma agonist, and morphine and morphiceptin, mu receptor agonists, on food consumption. All kappa agonists induced feeding when administered at 0800 h as did morphine (F 5.72; $p < 0.005$). Kappa agonists induced feeding within 2 hours whereas morphine took 3-4 hours to induce feeding. KC failed to induce feeding during the nocturnal feeding period (2000 and 0200 hours) and morphine suppressed feeding at these times. KC enhanced feeding at 1400 h, whereas morphine suppressed it. KC and morphine suppressed 24 h starvation induced feeding when food was made available immediately after injection and had no effect when food was presented 2 and 4 hours after injection. High doses of naloxone (5 mg/kg) suppressed KC (10 mg/kg) induced feeding while actually enhancing high dose morphine (25 mg/kg) induced feeding. Repeated daily injections of KC or morphine for 5 days resulted in an enhancement of the feeding response with initiation of feeding occurring earlier. The kappa-sigma agonist, butorphanol, markedly enhances feeding in rats at doses ten times lower than those required by morphine to initiate feeding. This butorphanol effect on feeding is highly resistant to naloxone's suppressive effect (doses up to 10 mg/kg). β-casomorphin, 10 µg to 75 µg ICV, failed to induce feeding whereas dynorphin, 10 µg ICV, was a potent inducer of feeding. The most parsimonous interpretation of these studies is that kappa agonists are endogenous initiators of feeding. The kappa receptors are maximally saturated at times of food deprivation and during spontaneous nocturnal feeding. The mu opiate receptors inhibit feeding due to their sedative effect and antagonism of this effect leads to enhancement of the feeding response. It is postulated that kappa opiate receptors represent an important component of the natural feeding drive.

206.5 NALOXONE AND NALTREXONE CHANGE FOOD SELECTION IN HUNGRY RATS UNDER CONDITIONS OF FAMILIARITY. S. Turkish* and S.J. Cooper* (SPON. B.J. Sahakian). Dept. of Psychol., Univ. of Birmingham, Birmingham B15 2TT, U.K.

Considerable evidence affirms that opiate antagonists, naloxone (NAL) and naltrexone (NALT), attenuate food intake in many species (Sanger, D.J., Appetite, 2: 193, 1981). Much less attention has been paid to their effects on food selection. In our model, both NAL and NALT markedly affected the choice between two foods in hungry rats, provided they were tested under conditions of familiarity. Both drugs acted selectively to reduce the palatability of the more preferred food, so that the food selection was diverted towards the less preferred food. Adult, male, hooded rats were given 15 min access to two foods: chocolate-coated cookies (Cadbury's), and Diet 41B food pellets. The pellets were familiar as the home cage diet. Food intake and the duration of feeding were recorded for each food, as well as a variety of non-feeding behaviors. Each subject was tested under one of 4 conditions:- 24h food-deprived + test familiarity, including the cookies as familiar food (DEP-FAM); food-deprived + test novelty, including the cookies as novel food (DEP-NOV); non-deprived + familiarity (NDEP-FAM); non-deprived + novelty (NDEP-NOV). Under the DEP-NOV condition, control rats showed a definite selection in favor of the food pellets. NAL (0.5 and 2 mg/kg, s.c.) did not alter this choice. However, under the DEP-FAM condition, control rats showed an exclusive choice of the highly palatable cookies, and ignored the food pellets altogether. Both NAL and NALT (0.5 and 2 mg/kg in each case) significantly reversed this food selection which was related to palatability (significant drug x food choice interactions, $p < 0.001$, in each case). Whilst both drugs reduced the intake and feeding duration for the palatable cookies, they increased the intake and feeding duration of the food pellets. In effect, the strong preference for the more palatable food evident in control rats tested under conditions of familiarity was sharply attenuated by NAL or NALT treatment. The opiate antagonists did not impair preference behavior or food discrimination per se, since the food selection in the DEP-NOV condition was not altered. No significant effect of NAL on feeding behavior was observed under either NDEP condition. However, NOV induced excessive grooming under both DEP and NDEP conditions. This grooming was significantly attenuated by NAL treatment. The reduction in novelty-induced grooming appeared to be independent of NAL's effect on feeding selection which was manifest under conditions of familiarity. Opiate antagonists may therefore exert separable effects on food selection and on behavioral responses elicited by novelty.

206.6 GLUCOPRIVATION-INDUCED RELEASE OF BRAIN NEUROTRANSMITTERS. J. O'Fallon and S. Ritter. Dept. of Animal Science and College of Veterinary Medicine, Washington State University, Pullman, Wa 99164.

Both in vivo and in vitro studies have demonstrated that norepinephrine (NE) release is increased in various brain regions during glucoprivation. Since increased feeding also occurs during glucoprivation, it has been suggested that a causal relationship may exist between the increased release of NE and the appearance of feeding behavior. However, we recently showed that the rate of NE release from hypothalamic neurons in vivo indeed reflects their supply of utilizable energy substrate (i.e., β-hydroxybutyrate or glucose), but that NE release and feeding behavior were dissociable under a number of circumstances. This suggests that increased NE release during glucoprivation might be an epiphenomenon, not causally related to the feeding response. If so, one might expect glucoprivation to alter release of other neurotransmitters which have thus far not been implicated in glucoprivic feeding. In order to test this hypothesis, we measured the effects of in vitro glucoprivation on release of tritiated putative neurotransmitters from brain tissue prisms. Mouse brains were dissected and prisms from cortex, cerebellum, hypothalamus, midbrain and pons-medulla were preincubated for 20 min in a buffer containing an appropriate inhibitor of transmitter degradation. Tissues were then incubated for 20 min with a tritium-labelled transmitter (NE, dopamine, serotonin, gaba-aminobutyric acid, β-alanine, D-aspartate or choline), and subsequently washed prior to transfer to superfusion chambers. In the chambers, the prisms were superfused with buffer solution containing glucose concentrations between 0 and 5 mM. Aliquots of the superfusate were collected over a 45 min period and counted. We found that in the absence of depolarizing agents, spontaneous release of most neurotransmitters was enhanced in the presence of low glucose concentrations. Aspartate was a notable exception. The magnitude of the effect varied across transmitters and the magnitude for a given transmitter was dependent upon brain region. Thus, our preliminary findings suggest that NE neurons may not be unique in their response to glucoprivation. In addition, our results indicate that glucose availability may significantly influence brain neurotransmission.

206.7 BRAIN DA-DEPLETION BLOCKADE OF HYPOTHALAMIC OBESITY DOES NOT OCCUR WITH SIMULTANEOUS BRAIN NE DEPLETION. D.V. Coscina, J.N. Nobrega, and J.J. Warsh. Sections of Biopsychology and Biochemical Psychiatry, Clarke Institute of Psychiatry, University of Toronto, Toronto, Ontario, Canada, M5T 1R8.

Past research has shown that damaging brain dopamine (DA) neurons by central injection 6-hydroxydopamine (6OHDA) disrupts many behaviors including feeding. However, conflicting reports exist over the ability of such 6OHDA treatment to block the over-eating and obesity which follow medial hypothalamic lesions (MHL). To address this issue, we injected groups of 6 adult female rats intracisternally once weekly for 3 consecutive weeks as follows: (1) 200 µg 6OHDA in 1% ascorbic acid vehicle 1 hr after 30 mg/kg desipramine (DMI) i.p.; (2) 240 µg 6OHDA in vehicle 1 hr after 50 mg/kg pargyline (PAR) i.p. (pretreatment on week 1 only); (3) vehicle after DMI as in (1); (4) vehicle after PAR as in (2). An additional 6 rats received no treatment (normal controls). Following 4 weeks of body weight and food intake measurements, all 4 injected groups received bilateral radiofrequency MHL. Body weights and food intakes were measured for an additional 5 weeks before sacrificing all rats for striatal and neocortical assays of endogenous DA, norepinephrine (NE) and serotonin (5HT) content. Compared to normal controls, MHL produced equivalent obesity (360-385% greater weight gain) in all groups except DMI-6OHDA rats (67% less gain than normals). Regardless of pretreatment, 6OHDA produced equivalent depletions of striatal (84-91%) and cortical (70-78%) DA without affecting 5HT. However, the DMI-6OHDA rats, who displayed no obesity after MHL, possessed normal levels of cortical NE. This contrasted with the PAR-6OHDA rats, who were obese after MHL, and possessed 72% depletion of cortical NE. MHL after either pretreatment plus vehicle was without long-term effect on striatal or cortical DA, NE and 5HT. Our findings show that selective depletion of brain DA is indeed capable of blocking the development of overeating and obesity after MHL. However, simultaneous depletion of brain DA and NE to comparable degrees produces no such blockade. These results resolve conflicting reports about the ability of central 6OHDA to block MHL obesity by showing that such blockade occurs only when brain DA depletion substantially exceeds that of brain NE. Such findings support previous suggestions of an important balance between brain DA and NE systems in the control of several behaviors. (Supported in part by MRC grant MA-6579 to D.V.C.).

206.8 THE EFFECTS OF BIOGENIC AMINES, VASOPRESSIN AND HYPERTONIC SALINE ON BLOOD PRESSURE AND DRINKING AT THE SUBFORNICAL ORGAN (SFO). J.B. Simpson and A. Josefiak*, Dept Psych., Univ. Washington 98195

The SFO is a circumventricular organ of the third ventricle which functions in fluid balance. Injections in rats of angiotensin II (AII) or cholinergic agonists there provoke correlated increases in water intake and blood pressure (Mangiapane and Simpson, AJP, 1980, 1982). We investigated neurochemicals which are endogenous to the SFO for effects on drinking and blood pressure.

Male, Long-Evans rats each received a single intracranial cannula for injection of one compound in a 0.5 ul volume into the central zone of the SFO. A chronic aortic catheter was used to monitor blood pressure in the conscious, unrestrained rodents.

Injection of the catecholamines, norepinephrine and dopamine, into SFO did not increase drinking and did not alter blood pressure. Prior injection of these amines did not affect the dipsogenic or pressor effects of AII at the SFO. In contrast, injection of serotonin (5-HT) provoked dose-dependent increases in aortic pressure and drinking. Onset of the pressor response always preceded the onset of drinking, both occurring within 1 min.

The peptide vasopressin (AVP),also endogenous to the SFO, was injected into another group of rats and, like 5-HT, produced dose-dependent increases in blood pressure and drinking. The pressor and dipsogenic effects of 5-HT and AVP, in terms of latencies and elicited behavior, were indistinguishable from the effects of acetylcholine and AII applied to the SFO.

We also studied water intake following SFO application of hypertonic NaCl. Lesions of the SFO impair both the AVP secretion and the drinking following systemic hypertonic NaCl (Mangiapane, et al, Fed Proc, 1982), and Thrasher et al (AJP, 1980) suggest that osmoreceptors for thirst and AVP secretion are within one of the forebrain circumventricular organs. We found, however, that SFO application of graded doses of 1.0 ul of NaCl (.153 M, .156 M, .162 M, and .174 M) did not provoke drinking. In addition an 11 min infusion of .156 M or .162 M NaCl at 0.18 ul/min also did not cause water intake.

Catecholamines, which are endogenous to the SFO, apparently do not function in control of drinking or blood pressure, whereas the indoleamine 5-HT does. Likewise, AVP application to the SFO provokes dipsogenic and pressor effects in analogous fashion to AII injections. Hypertonic saline does not affect water intake at the SFO, suggesting that this circumventricular organ does not harbor osmoreceptors for drinking.

Supported by HL 21800.

206.9 Fourth ventricular alloxan injection causes feeding but not hyperglycemia in rats. Sue Ritter and Mary Strang, Dept. of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164 and University of Iowa Hospital, Iowa City, IA 52242.

Intracerebroventricular administration of the antiglycolytic agents 5-thioglucose (5TG) and 2-deoxy-D-glucose stimulates feeding and sympathoadrenal discharge. Recently, the diabetogenic agent, alloxan, has been reported to inhibit glucose metabolism in cultured fibroblasts and pancreatic islets. Assuming that alloxan might also exert an antiglycolytic effect on brain glucoreceptor cells controlling feeding and sympathoadrenal activity, we measured food intake and blood glucose in eight rats immediately following IV ventricular alloxan injections (0,5,10, 15 and 20 μg in 5 μl saline, pH 3.0). Compared to control, 4-hr food intakes were significantly elevated after 10, 15 and 20 μg of alloxan ($p<.01$). At these doses, rats ate 2.5 ± 0.5, 2.5 ± 0.5 and 2.0 ± 0.8 g of food above control levels, respectively. Intake after saline injection was 0.6 ± 0.3 g of food. In contrast to feeding, blood glucose was not altered by any dose of alloxan, even though a typical hyperglycemic response was obtained when 5TG (90 μg in 5 μl) was injected subsequently through the same cannula.

The ability of alloxan to stimulate feeding without evoking hyperglycemia is consistent with our previous observation that a higher alloxan dose (40 μg) permanently impairs the glucoprivic feeding response without altering the blood glucose response. These data support the hypothesis that the feeding and sympathoadrenal responses to glucoprivation are mediated by separate cell populations differentially susceptible to alloxan's effects. The possibility that intraventricular alloxan stimulates feeding by inhibition of glucose metabolism in brain glucoreceptor cells is a reasonable hypothesis but requires further experimental support.

206.10 ENHANCED INTAKE OF PREFERRED FOODS FOLLOWING INJECTION OF CAPSAICIN INTO THE CAUDAL BRAINSTEM. Elizabeth H. South and Robert C. Ritter. WOI Regional Program in Veterinary Medicine, University of Idaho, Moscow, ID 83843, and Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Thermal lesions of the area postrema (AP) and the adjacent nucleus of the solitary tract (NST) cause increased intake of preferred foods and solutions (Edwards & Ritter, Brain Res. 216:265, 1981) and exaggerated drinking in response to angiotensin (AII) and extracellular thirst challenges (Edwards & Ritter, Neurosci. Abst., 1980). In addition AP-NST lesions usually produce a chronic body weight reduction (Hyde & Miselis, Neurosci. Abst., 1980). Therefore, it appears this small brainstem region participates in several ingestive and body weight controls.

To determine the role of neurochemically discrete cell populations in the behavioral and physiological alterations produced by thermal AP-NST lesions, we have injected the neurotoxins, capsaicin, 6-hydroxydopamine (6OHDA), and 5,7 dihydroxytryptamine (5,7DHT), into the region of the AP and NST. None of the neurotoxin treatments altered drinking in response to AII, feeding in response to 2-deoxy-D-glucose, or caused permanent reduction of body weight.

The capsaicin-injected rats did, however, eat an average of 58% (6.5±0.5 gr, n=12) more than the control rats (4.1±0.3 gr, n=20) when given a preferred food (cookies) for a 30-min test period. The intake of preferred foods was not increased in 6OHDA- or 5,7DHT-treated rats. Also capsaicin-treated rats fed ad libitum rat chow and cookies for 24-hr periods significantly suppressed rat chow intake. They consumed an average of 93±1% of their total calories in cookies as compared to 74±3% for the controls. There was no significant difference in total calorie intake or in water intake between capsaicin-treated and control rats. Thus pretreatment with capsaicin, a neurotoxin that damages substance P-containing sensory neurons, enhances preferred food consumption and alters diet choice in rats. These findings parallel those found following thermal AP-NST lesions. However, the capsaicin-induced enhancement of preferred food intake occurs in the absence of the alteration in drinking behavior and the chronic weight reduction observed after thermal lesions. Capsaicin, therefore, allows us to selectively examine the cell populations involved in one facet of the AP-NST lesions syndrome.

206.11 PRESENCE OF PREFERRED FOOD OR GLUCOSE CAUSES EXAGGERATED ALTERATION OF DIET CHOICE IN AREA POSTREMA-LESIONED RATS. G.L. Edwards and R.C. Ritter. WOI Regional Program in Veterinary Medicine, University of Idaho, Moscow, ID 83843 and Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Rats with lesions of the area postrema (AP) and adjacent nucleus of the solitary tract (NST) overconsume preferred foods and solutions during short tests. The fact that lesioned and control rats eat similar amounts of laboratory chow ad lib. or after food deprivation suggests the AP-NST lesions have a specific effect on intake driven by palatability.

In order to determine whether the chronic presence of a preferred substance altered caloric intake or diet choice, we offered AP-lesioned and control rats a choice between a preferred substance and rat chow as their daily ration. The animals were offered chow alone, or together with saccharin (0.13%), 5%, 15%, 35% glucose, or cookies for periods of 5 days each. Total caloric consumption by the two groups was not different under any condition. However, when cookies, 15%, or 35% glucose were available, lesioned rats took significantly greater portions of their total intake from these substances than did control rats. For example, in the presence of 35% glucose, lesioned rats took 93±1% of their calories as glucose whereas controls took only 76±4% of their calories as glucose. Lesioned rats also exhibited a greater reduction in lab chow intake when calorically dense glucose solutions or cookies were available. For example, in the presence of 35% glucose, control intake of lab chow averaged 7.5±1.0 g/day while lesioned rats ate only 2.1±0.3 g/day. Rat chow consumption of lesioned and control rats did not differ in the presence of 5% glucose or saccharin.

These data suggest that enhanced responsiveness to preferred foods in AP-NST lesioned rats does not alter caloric intake but does influence the choice of calorie source.

207.1 EVIDENCE FOR GANGLION CELL SPECIFICITY OF SEVERAL MONOCLONAL ANTIBODIES THAT BIND TO CHICK RETINA. V. Lemmon and S.C. McLoon. Dept. of Anatomy, Medical University of South Carolina, Charleston, S.C. 29425.

We are interested in studying the development of retinofugal projections both in vivo and in vitro. For many of our studies it would be most useful to identify specific classes of cells and even subclasses in the developing retina and central visual nuclei. Over the past few years it has been recognized that the monoclonal antibody technique can be used to produce cell specific markers. Several antibodies have been made which appear to be specific for elements of the inner plexiform layer, ganglion cell layer and/or optic fiber layer in the developing chick retina. In order to further define the cell selectivity of these antibodies we have lesioned the tectum of early embryos which causes a subsequent loss of most of the retinal ganglion cells. These retinas were examined for changes in the binding of our different antibodies.

Chick embryos at 3 days of incubation were transferred to embryo culture chambers to facilitate access. The right tectum was ablated completely with an electrocautery. On E17 the embryos were fixed and the left retina of each was sectioned on a cryostat. The sections were processed for immunohistochemistry using each of the antibodies. Retinas from embryos without lesions were processed in the same manner to serve as controls.

Nissl stained sections of the retinas from embryos with tectal lesions showed an extensive loss of cells from the ganglion cell layer compared to normal retinas. Only 6% of the ganglion cells remained, and they represent a unique class which projects to the thalamus and pretectum. The remainder of the neurons in the ganglion cell layer are displaced amacrine cells. Antibody Ret 2, which binds to the optic fiber layer (OFL) in normal retinas, was undetectable in retinas of the lesioned embryos. Antibody Ret 3 binds to the inner plexiform layer (IPL), ganglion cell layer, and OFL in normal retinas and staining with it was also completely abolished in the experimental retinas. Antibody Ret 5 shows staining in the OFL, IPL and the outer plexiform layer (OPL) in normal retinas but only bound to the OPL in the retina of lesioned embryos. Several antibodies that bound to the entire retina showed the same staining pattern in the experimental retinas.

It appears that antibody Ret 2 and 3 may bind specifically to chick retinal ganglion cells. Furthermore, they may represent binding to a specific class or classes of ganglion cells since they did not appear to stain the 6% of the ganglion cells which survive tectal ablations. Nor did they appear to stain the displaced ganglion cells. Although antibody Ret 5 shows some ganglion cell staining characteristics, it clearly cross-reacts with other retinal elements.

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207.2 ^{3}H-GABA UPTAKE SELECTIVELY LABELS IDENTIFIABLE NEURONS IN THE LEECH CNS. H.T. CLINE* (SPON: G.S. Stent). Graduate Group in Neurobiology, Univ. of California, Berkeley, Ca. 94720.

Isolated adult ganglia and embryonic nerve cord from the leech can convert glutamate to gamma-aminobutyric acid (GABA), indicating the presence of glutamic acid decarboxylase, an enzyme concentrated in neurons which synthesize GABA for neurotransmission. In order to locate the putative GABA-ergic neurons in the leech nerve cord, midbody ganglia from adult specimens of Haementeria ghiliani and Hirudo medicinalis were incubated with uM concentrations of ^{3}H-GABA, embedded in plastic, sectioned and processed for autoradiography. Reconstructions of the ganglia from serial sections reveals cell bodies which selectively accumulate exogenous ^{3}H-GABA. In Haementeria ghiliani a specific subset of 28-33 cell bodies per ganglion label with ^{3}H-GABA. There are variations in the labeling pattern between ganglia taken from different segments, comparable to the ganglionic variations in the monoamine-containing neurons, but ganglia from any given segment have the same pattern of labeled cell bodies from preparation to preparation. The labeled cell bodies are distributed in all 6 glial packets; most of the cell bodies are bilaterally paired, although there are some apparently unpaired ^{3}H-labeled neurons in the medial packets.

The neuropil and interganglionic connective also accumulate ^{3}H-GABA in reproducible patterns. Neurites from 2 pairs of labeled cell bodies in the dorsal surface of the ganglion can easily be traced across the neuropil in the anterior commissure by their accumulation of grains. Lines of grains can be followed along the longitudinal fiber tracts in the neuropil and into the lateral axon bundles of the connective. Transverse sections through the connective reveal bilaterally symmetric spots of grains in the lateral axon bundles which may be processes of some of the bilaterally paired ^{3}H-GABA-labeled neurons in the ganglion. In addition, there is a spot of label in Faivre's nerve, the unpaired medial connective. Incubation at 4^{o}C abolishes labeling in the cell bodies, neuropil and connective. Glia accumulate ^{3}H-GABA under the incubation conditions, but this does not interfere with the interpretation of the autoradiograms because there are only 6 large glial cells per ganglion, and they are easily distinguished from the neuronal cell bodies in sectioned material.

Neurons which accumulate ^{3}H-GABA have been tentatively identified in the living ganglion, injected with Lucifer Yellow or HRP prior to GABA incubation and autoradiography, and then sectioned material was examined for the presence of both markers.

207.3 MONOCLONAL ANTIBODIES AS PROBES OF THE LEECH NERVOUS SYSTEM. R. McKay, S. Hockfield, J. Johansen, L. Kleina, I. Thompson Cold Spring Harbor Lab, P.O. Box 100, Cold Spring Harbor, NY 11724

With a surprisingly high frequency monoclonal antibodies raised against the leech nerve cord bind to antigens present in small subsets of the four hundred neurons in the typical midbody ganglion of the leech (Zipser and McKay, 1981, Nature 289, 549). The large number of antigens which have a restricted neuronal distribution suggests that each neuron in the midbody ganglion could be molecularly unique.

An advantage of the leech for this immuno-histochemical study was that a single cell type could be identified morphologically. Using this anatomical feature we have dissected single cell types and with Western blots analyzed the cellular distribution of specific antigens. EM studies using these antibodies show that (1) axons occupy stereotyped locations in fiber tracts and (2) many different subcellular organelles carry these specific antigens. These general studies suggest that many rather than a single specific feature of the connectivity and physiology of a particular class of neuron may be a consequence of the presence of specific molecules in that class of cell.

One antibody (Lan3-2) binds to both medial and lateral nociceptive (N) cell bodies in midbody ganglia (7-19) of H. marmorata. However in ganglia 5, 6, 20 and 21 only a single cell body binds Lan3-8. Electrophysiology on H. medicinalis confirms that ganglia 5 and 6 have only a single N cell. Double-labeling with lucifer yellow and a second antibody which recognizes only the lateral N cell in Hirudo suggests that the single N cell in ganglia 5 and 6 is the medial N cell and the cell in 20 and 21 the lateral N cell. Immunoelectron microscopy shows that the Lan3-2 antigen binding sites are found on the surface of nociceptive cells and central axons. The axons carrying this surface antigen travel in a stereotyped, antigenically homogenous group in the leech fiber tracts. These peripheral cells that express this antigen do so from the earliest stages of neurite growth. Additionally this antibody binds to three bands on Western blots of leech neural proteins. Three different species of leech each with specific technical advantages were used to obtain this information. From these results we propose that the surface proteins recognized by this antibody may play a role in the mechanisms which guide the precise outgrowth of these axons during the development of the leech nervous system.

207.4 MONOCLONAL ANTIBODIES RECOGNIZE SUBSETS OF NEURONS IN CAT SPINAL CORD AND BRAIN, S. Hockfield and R. McKay, Cold Spring Harbor Lab, Cold Spring Harbor, N.Y. 11724.

Molecular differences among neurons may play a role in the specificity of neuronal connections and account for the physiological and anatomical differences among neurons. Monoclonal antibodies have been generated that recognize molecularly homogenous subsets of neurons.

Monoclonal antibodies were raised by immunizing mice with homogenized, formaldehyde fixed grey matter of the adult cat cervical spinal cord. Hybridoma lines were screened immunohistochemically on 50 m thick vibratome sections of fixed cat spinal cord and other areas of the CNS using HRP histochemistry. Of 800 cell lines screened, 47 secreted antibodies that bound to spinal cord sections. On the basis of the neural elements recognized, the antibodies can be grouped into four classes: 1. axons only; 2. axons and cell bodies; 3. cell bodies only; and 4. glial cells. Of the five neuronal antibodies cloned and studied in more detail, each recognizes a subpopulation of neural elements throughout the CNS; none stains the CNS uniformly.

Two antibodies which recognize projection neurons provide an example of the specific staining we observe. The morphology of neurons stained by antibodies Cat-201 and Cat-301 match that of projection neurons in the spinal cord, but the distribution of Cat-301 positive cells is more extensive than that of Cat-201. Similarly, Cat-201 recognizes neurons in only a few brainstem nuclei while Cat-301 recognizes neurons in many more locations. As an example of the specificity of antigen distribution, in the pons Cat-201 only binds to neurons in the trigeminal motor nucleus while Cat-301 binds to many neurons in the trigeminal motor and main sensory nuclei, the facial motor nucleus, the vestibular nuclei, and the reticular formation, but to few neurons in the periventricular grey matter. EM localization shows that Cat-301 antibody binding sites are found around synaptic profiles, that is, HRP-bound antibody is found along the surface of the cell body and proximal dendrites but is excluded from the immediate region of synaptic junctions, often forming a cup of stain around the presynaptic profile.

These monoclonal antibodies recognize molecular differences which distinguish neurons from one another. None of our antibodies binds uniformly to all the neurons in the CNS, indicating that the number of different molecular features among neurons is likely to be large.

207.5 SYMMETRICAL GIANT NEURONS IN ASYMMETRICAL MOLLUSCAN GANGLIA. Douglas Munoz, Peter A. Pawson and Ronald Chase. Dept. Biology, McGill University, Montreal, Quebec, H3A 1B1.

The central nervous system of gastropod molluscs is composed of a collection of ganglia that have been subject to extensive change during evolution. One way to reconstruct the evolutionary history is to examine cellular homologies. For example, in Aplysia, the abdominal neuron R2 and the pleural neuron LP1 presently reside in non-paired asymmetrical ganglia, but they share properties which suggest an evolutionary origin in bilaterally symmetrical ganglia (Hughes and Tauc, 1963). We have studied a similar situation in the terrestrial snail Achatina fulica where the neurons RPr1, in the right parietal ganglion, and V1, in the visceral ganglion, constitute an homologous pair. The evidence for this is as follows:

1) Both cells have unusually large soma diameters (RPr1, 194-345μ; V1, 219-375μ). In a given animal, the sizes are closely matched (mean difference, 24 ± 6μ).
2) HRP injections reveal extensive axonal projections, with each cell appearing as a mirror image of the other.
3) The electrical properties are very similar, e.g. resting potential, input resistance, time constant, anomalous rectification, and the presence of a large (15-20mV) hyperpolarization after the action potential.
4) There is a high degree of synchrony in spontaneous PSPs.
5) Tactile stimulation of the skin over an extensive area, or electrical stimulation of peripheral nerves or connectives, evokes synchronous compound EPSPs. Responses are consistently larger in the ipsilateral cell.
6) Stimulation of the pre-synaptic neuron V2 evokes facilitating monosynaptic EPSPs in both cells.
7) Direct stimulation of either cell leads to muscle contractions in the foot and mantle. The responses persist during suppression of chemical synaptic transmission in the CNS.
8) The motor responses of each cell show both short-term facilitation and long-term habituation.
9) Application of serotonin, octopamine or GABA produces depolarization in both cells; ACh is hyperpolarizing; glycine and glutamic acid are ineffective in both cells.

The foregoing results suggest that V1 and RPr1 are an homologous pair. If so, they probably arose in bilaterally symmetrical ganglia of an early gastropod. Their contemporary locations in Achatina suggest an origin in the once symmetrical intestinal ganglia. This interpretation is consistent with the previously stated hypothesis (Haeckel, 1913) that the supra-intestinal ganglion has fused with the right parietal ganglion and the sub-intestinal ganglion has fused with the visceral ganglion.

207.6 SEROTONIN ANTIBODY STAINING AND LUCIFER YELLOW INJECTION REVEAL DIFFERENT FEATURES OF THE NORMAL AND REGENERATE MORPHOLOGY OF AN IDENTIFIED NEURON. A. D. Murphy, D. L. Barker and S. B. Kater. Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Tonic activity in the serotonergic cerebral cells (SCCs) of Helisoma can initiate or modulate feeding motor output from the buccal ganglia. In addition, bath applications of 5-HT mimic the effects of SCC activity on feeding motor output (Granzow and Kater, Neurosci. 2:1049-1063, 1977). In the current study we axotomized the SCCs by crushing the cerebro-buccal connectives (CBCs) and allowed them to regenerate in vitro in modified L-15 culture medium. After approximately one week of culture, normal effects of SCC activity on the feeding motor output of the buccal ganglia were restored. Despite extensive surveys, however, direct synaptic connections from the SCCs to buccal neurons have never been observed in either normal or regenerate preparations. These observations suggest a humoral mechanism for the effects of SCC activity on "feeding."

Lucifer Yellow stains of the SCCs displayed relatively unadorned stick-like axons coursing through the buccal ganglia without elaborations in the neuropile and exiting buccal nerves. The 5-HT-antibody technique, on the other hand, revealed very fine processes arising from these axons and elaborating varicosities (possible humoral release sites) in or near the ganglionic and nerve sheathes. This staining technique also allowed us to see fine processes on distant targets (e.g., the gut and salivary glands) which have not been seen using Lucifer Yellow staining. 5-HT antibody staining has also provided complementary information to that provided by Lucifer Yellow staining of SCCs during regeneration. The growth patterns of regenerating SCCs following CBC crush is very distinctive and reproducible. The 5-HT antibody technique allowed us to examine the relationship between sprouts from the proximal axon stumps and the surviving distal axon segment. It also disclosed that the severed distal segment has at least a limited capacity to extend sprouts of its own. Lucifer Yellow staining provides a very useful but only partial sketch of a rich neuronal landscape. Antibodies made to neural transmitters such as 5-HT can provide additional brush strokes to the canvas. (Supported by NIH grants NS 15350 and AM 19858.)

207.7 INTRANEURONAL INJECTION OF HORSERADISH PEROXIDASE LABELS GLIAL CELLS ASSOCIATED WITH AN IDENTIFIED APLYSIA NEURON Ronald S. Goldstein*, Klaude R. Weiss, and James H. Schwartz. Center for Neurobiology and Behavior, Columbia Univ. College of P&S, New York, 10032

After injecting the cell body of the giant serotonergic neuron (GCN) in the cerebral ganglion of Aplysia with horseradish peroxidase (HRP), we found that some glial cells were labeled along the cerebrobuccal connective and posterior lip nerve, the two nerves that contain the major axon branches of the injected neuron. Starting at a distance of about 3 mm from the site of the injection, only glial cells in the nerve that are close to an axon branch of GCN contained HRP. Labeling appears to be selective because, at most, only one out of three glial cells were labeled within an area of 20 μm of the axon. Moreover, no HRP was detected in any of the many glial cells within the cerebral ganglion which come into intimate contact with the GCN's initial segment and cell body. Reaction product diffusely labeled the cytoplasm of glial cell bodies and processes after intraneuronal injection of HRP. This distribution was markedly different from that observed in glial cells after uptake of HRP from the extracellular space; HRP, presumed to be taken up by endocytosis, was found to be localized to vesicles, tubules, and multivesicular bodies. Labeling of adaxonal glia is not restricted to GCN, but also occurs when HRP is injected into R2, the giant neuron of the abdominal ganglion and B2, a motor neuron of the buccal ganglion.

We presume that the transfer of HRP reflects a functional connection between the axon of the neuron and special glia cells that permit passage of the 40,000 molecular weight protein. We have not observed any junctions between axons and glial cells in Aplysia, but morphological connections with glia have been described in other invertebrate axons. While the mechanisms of transfer have not yet been characterized, the ability to identify glial cells specialized for individual neurons could provide the opportunity of determining how a neuron relates specifically to a group of glial cells.

207.8 BINDING SITES FOR Fc IgG IN RABBIT CILIARY PROCESSES, N. S. Peress V. A. Roxburgh* and M. C. Gelfand*. Lab. of Neuropathology, V. A. M. C. Northport, NY 11768.

Binding sites for IgG and C have been defined in renal glomeruli and choroid plexus. These structures are analogous in form and function to the processes of the ciliary body and like the ciliary processes exhibit immune complex entrapment in Systemic Lupus Erythematosus (SLE), murine SLE and experimental serum sickness. The present study demonstrates the presence of binding activity for the Fc fragment of IgG selectively on the processes of the ciliary body of adult rabbit eyes. Other regions of the eye including the cornea, iris, choroid and retinae were negative. Binding activity was demonstrated by an in vitro assay employing IgG-coated sheep red blood cells (IgGEA) and frozen sections of longitudinally split rabbit eye. The eyes of 8 normal adult rabbits, a single pregnant rabbit and 2 of her fetuses were studied. All slides were code numbered and examined by an independent observer who counted the number of RBCs on each of 3 randomly chosen HPFs of ciliary processes, retinae, choroid, iris and cornea. For each animal, two slides were counted for each area and reagent. The intensity of reagent binding to a particular ocular tissue was graded from 0 to +6 (a score of trace correlated with 1-10 RBC/HPF, 1+ with 11-20 RBC/HPF, 2+ with 21-40 RBC/HPF, 3+ with 41-60 RBC/HPF, 4+ with 61-80 RBC/HPF, 5+ with 81-100 RBC/HPF and 6+ with $>$ 100 RBC/HPF) based upon cell counts. All adult rabbit eyes exhibited adherence of the IgGEA reagent to the epithelial layers of the ciliary processes which was 6+ in 5, 5+ in 1, 4+ in 1 and 2+ in 2. In contrast both fetal eyes exhibited only trace to 1+ adherence of the IgGEA reagent to the epithelium of the ciliary processes. Adult and fetal ciliary IgGEA binding was totally blocked by 4.0 mg/ml IgG, aggregated and non-aggregated, but not by similar concentration of Albumin or the $F(ab')_2$ fragment of IgG. In the adult eye, approximately 50% blocking was achieved at the 0.4 mg/ml concentration of aggregated IgG. Therefore the calculated affinity of the binding sites for IgG (K_D) is approximately $2x10^{-9}$M. Specificity was further suggested by the absence of adherence of both non-sensitized RBCs and of IgM sensitized RBCs. These studies therefore demonstrate maturation dependent binding sites for Fc IgG on rabbit ciliary processes. These IgG binding sites may be important determinants of this tissue's selective entrapment of circulating immune components. They may also function in clearing the aqueous humor.

207.9 LAMINA SPECIFIC MONOCLONAL ANTIBODIES STAIN RETINAL NEURONS MAINTAINED IN VITRO. M.B. Eschmann*, D. Witte*, C. Gottlieb* and D. Gottlieb. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

We have recently described monoclonal antibodies which bind to restricted layers of the chick retina (Lemmon and Gottlieb, J. Neurosci., in press). One of these, RET4, recognizes an antigen found in the optic fiber layer, ganglion cell layer, inner plexiform layer and the inner half of the nuclear layer. All other parts of the retina are unstained by RET4.

Cultures of dispersed retinal cells were made from seven-day embryonic retinas and maintained in vitro on polylysine-coated coverslips for one to four days. When such cultures were stained by indirect immunofluorescence with RET4 antibody strong fluorescence was seen on some cells; many others were clearly negative. The stain was clearly associated with the plasma membrane. This result was obtained whether or not the cells were fixed prior to staining. Since the antigen can be detected on living cells, it must be present on the cell surface. RET4 positive cells assume a variety of forms. Some are multipolar, with processes at least 100 μ long. Others are bipolar. The RET4 negative cells also have a variety of forms. Another monoclonal antibody 224-1A6-A1 has been described (Lemmon et al., Dev. Brain Res. 3:349) which stains all layers of the retina. This antibody stains nearly all of the cells in dispersed cell cultures of the retina; the stain is associated with the plasma membrane. It stains living cells indicating the antigen is on the cell surface. A number of small round cells are not stained.

Two other monoclonal antibodies RET1 and RET5 were studied in this manner. RET1 is specific for the optic fiber layer; RET5 binds selectively to the inner and outer plexiform layers. Both antigens are present in the seven-day retina. We could not detect these antigens in dispersed cell cultures. Two explanations for this result are: 1) the antibodies fail to stain because the antigens are inaccessible in cultured cells or 2) the antigens fail to be expressed in dispersed cell cultures. We are currently trying to decide among these explanations.

Our results show that monoclonal antibodies can help identify retinal cells in vitro according to their layer of origin in vivo. Since several of these antibodies are against cell surface antigens, they may also be useful for selecting populations of cells for culture.

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207.10 ULTRASTRUCTURAL ANALYSIS OF DORSAL AREA OF TURTLE DORSAL VENTRICULAR RIDGE, INCLUDING COMMENTS ON CELL CLUSTERS. J.C. Weiss Dept. of Anatomy, Univ. of Chicago, Chicago, Il 60637

Dorsal ventricular ridge (DVR) is a subcortical structure receiving sensory information from the thalamus in reptiles. It includes four cytoarchitectonic areas in the turtle Pseudemys scripta, each characterized by distinct thalamic projections. The areas contain four concentric zones distinguished by the distribution of spiny and aspiny neurons. A marked feature is the presence of neuron clusters with touching somata. This is an electron microscoic study of dorsal area, which receives its thalamic input from the tectorecipient nucleus rotundus.

In the ependymal cell layer, tanycyte tails extend into zones 2 and 4 (Z2 and Z4) and cytologically resemble protoplasmic astrocytes. 23 synapses, with mainly asymmetric active zones and containing round synaptic vesicles, were observed on astrocytic processes in Z2 and Z4. Zone 1 (Z1) neurons have fusiform somata whose long axes parallel the ventricular surface. Their nuclei contain coarse, but finely clumped chromatin; the cytoplasm contains rough endoplasmic reticulum (RER) located primarily in Nissl bodies, lipofuchin granules, multivesicular bodies, extensive arrays of Golgi apparatus, and many mitochondria. Synapses occur mainly on dendritic spines of Z1 neurons, less frequently on dendritic shafts, and infrequently on somata. The majority (60/67) had round synaptic vesicles and asymmetric active zones. In contrast to Z1, neurons in Z2 and Z4 have large amounts of free RER giving their cytoplasm an electron dense quality. Synapses occur mainly on spines and shafts of Z2 and Z4 neurons. As in Z1, the majority (1270/1428) contain round synaptic vesicles and involve asymmetric active zones. Z2 and Z4 contain clusters of neurons distributed among isolated neurons; clusters are larger and more frequent in Z2. Protoplasmic and fibrous astrocytic processes, axon boutons, dendrites, and axon fasicles surround the clusters. Though less numerous, the same structures occur inside the clusters. Most synapses inside clusters had round synaptic vesicles, asymmetric active zones and occured mainly on spines; asymmetric and symmetric synapses were also seen on dendrites and somata. Some neurons in clusters have somata whose plasma membrane are in direct apposition. In contrast to snake DVR, no nexūs were seen. These observations lead to the speculation that entire clusters or single cells within clusters may be sequestered from the surrounding environment by interposition or retraction of astrocytic processes (Hatten and Tweedle, 1982, Brain Res. Bull. 87,197), possibly through the action of synapses on astrocytic processes. (Supported by PHS Grant NS12518)

207.11 CHRONIC GROWTH OF NG108-15 CELLS WITH DIAZEPAM ALTERS DNA PATTERNS IN THE NUCLEUS M. D. Dibner, R. Z. Lockart*, R. A. Lampe* and K. M. Pezzella*. Central Research and Development Department, E.I. Du Pont Glenolden Laboratory, Glenolden, PA 19036.

Neuroblastoma x glioma hybrid cells (NG108-15) possess large numbers of the peripheral-type benzodiazepine receptor. We examined cells grown for one week in medium containing 5% fetal calf serum and 10 μM diazepam. Growth of cells with diazepam led to minimal alterations in cell growth and gross morphology. In contrast, there were significant alterations in nuclear DNA patterns. Cells were fixed, stained according to the Feulgen procedure, then scanned and analyzed using a computer-assisted video monitoring system. This system objectively and quantitatively compared the images of the nuclei in treated and control untreated cells. Benzodiazepine-treated cells were discriminated from control cells with 97% accuracy by use of three features. One was a difference in DNA staining intensity and two were measures of DNA texture. Growth of cells with diazepam for 1, 4 or 7 days yielded a time-dependent change in these staining characteristics. Also, growth for 7 days with 1 nM, 100 nM or 10 μM diazepam showed a dose-dependent change in nuclear staining characteristics. In one experiment, differentiation of NG108-15 cells by growth for one week with 1 mM dibutyryl cyclic AMP led to gross morphologic alterations in whole cells as well as changes in DNA patterns similar to those seen with diazepam. In a second experiment, these changes were less pronounced. We are currently assessing the involvement of the benzodiazepine receptors in these phenomena and hope to further characterize the morphologic changes in these cells following treatment with benzodiazepines.

208.1 **QUANTITATIVE ANALYSIS OF DOPAMINE RECEPTOR SUBTYPES NOT LINKED TO ADENYLATE CYCLASE ACTIVATION IN RAT STRIATUM**

Rita M. Huff & Perry B. Molinoff. Dept. Pharmacology, Univ. of Penn., Philadelphia, PA 19104

A means of quantitatively assaying two classes of dopamine receptors in the striatum that are not linked to activation of dopamine sensitive adenylate cyclase has been developed. The binding of ^{3}H-domperidone and ^{3}H-spiroperidol, reported to be selective ligands for D-2 receptors, was examined in membranes prepared from rat striatum. Scatchard analysis of the binding of ^{3}H-domperidone resulted in markedly curvilinear plots consistent with the presence of multiple classes of binding sites. Nonlinear regression analysis of untransformed data showed that the curvature was best explained by the presence of two populations of binding sites; 21% of the sites had a K_d value of 130 pM and 79% of the sites had a K_d value of 1300 pM. In contrast, the binding of ^{3}H-spiroperidol appeared to be to a single class of receptors as indicated by linear Scatchard plots and Hill coefficients equal to one. Studies of the inhibition of the binding of ^{3}H-spiroperidol by a number of competing ligands, including domperidone, sulpiride, and dopamine in the presence of GTP, was consistent with the interaction of these agents with two classes of binding sites. GTP was included in studies with agonists to avoid problems due to formation of a high affinity complex with a guanine nucleotide regulatory binding component. Hofstee plots of these data, analyzed by a computer assisted iterative method, gave relative proportions of two classes of binding sites along with the affinities of these sites for the drug in question.

Analysis of the inhibition of the binding of ^{3}H-spiroperidol by 6 competing ligands gave the same relative proportion for two classes of sites as previously determined by analysis of the binding of ^{3}H-domperidone. In addition, the two classes of receptors labelled with ^{3}H-spiroperidol had affinities for domperidone (93 pM and 1800 pM) which were nearly the same as the K_d values determined directly from analysis of ^{3}H-domperidone binding isotherms. Furthermore, the capacity of the binding sites for ^{3}H-spiroperidol was equal to the total capacity of binding sites for ^{3}H-domperidone. These findings suggest that the two radioligands bind to the same two classes of binding sites. A comparison of the properties of binding sites for ^{3}H-spiroperidol in the frontal cortex to those of the two classes of binding sites present in the caudate suggest that neither of the two classes of sites are receptors for serotonin. The approach described will make it possible to assess the effect of physiological or pharmacological manipulations on the densities or properties of subtypes of dopamine receptors. (This work was supported by USPSH NS 18591).

208.2 APPARENT IRREVERSIBLE MODIFICATION OF DOPAMINE RECEPTORS BY EEDQ: BEHAVIORAL AND RADIOLIGAND BINDING STUDIES.

M.W. Hamblin and Ian Creese. Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla, CA 92093.

N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an irreversible alpha-adrenergic antagonist, also acts as a potent and longlasting in vivo antagonist of D-2 dopamine receptors after peripheral administration to rats. Animals given EEDQ 3-10 mg/kg i.p. exhibit catalepsy and greatly reduced apomorphine induced stereotypy, behavioral effects associated with D-2 dopamine receptor blockade. These effects are apparent up to 4 days after drug administration, with scores returning to control levels by day 7. In vitro receptor binding assays of striatal membrane preparations from these animals using the radioligand ^{3}H-spiperone directly demonstrate that EEDQ is a potent D-2 dopamine receptor antagonist. This antagonism proceeds via a reduction in D-2 receptor Bmax, with no change in the observed K_D for ^{3}H-spiperone, and is resistant to extensive washing of the membrane preparation after in vivo EEDQ exposure. These observations and the known chemical reactivity of EEDQ suggest that EEDQ inhibition of D-2 receptors is irreversible. Administration of behaviorally active doses of EEDQ effect a reduction of 50-85% in D-2 receptor number. Recovery of these losses, to levels 75% of that of control ^{3}H-spiperone binding values at 7 days after administration of 10 mg/kg, parallels the recovery from catalepsy and blocked apomorphine induced stereotypy. These doses of EEDQ also reduce binding of ^{3}H-flupentixol to D-1 and ^{3}H-dopamine to D-3 type dopaminergic binding sites, putative dopamine receptors with no known behavioral correlates. Recovery of radioligand binding to D-1 and D-3 sites also occurs rapidly. Because of the apparent covalent nature of its interaction with dopamine receptors and because of its activity after peripheral administration, EEDQ may prove useful in the study of the function and turnover of multiple dopamine receptor subtypes.

208.3 ACUTE RESERPINE MIMICS THE EFFECTS OF NIGROSTRIATAL 6-HYDROXYDOPAMINE LESIONS ON "D-3" SPECIFIC ^{3}H-DOPAMINE BINDING IN RAT STRIATUM.

S.E. Leff, M.W. Hamblin and Ian Creese. Dept. of Neurosciences, Univ. of California, San Diego, Sch. of Med., La Jolla, CA 92093.

In the attempt to understand the neuroanatomical localization of multiple dopamine receptor subtypes in rat brain, selective 6-hydroxydopamine lesions of the nigrostriatal tract have been used to remove this afferent to the striatum. Most reports agree that this lesion induces a progressive increase in D-2 dopamine receptors. Some reports have indicated that another class of dopaminergic binding sites ("D-3") identified as having high (K_D~nM) agonist affinity but low butyrophenone antagonist (K_i~μM) affinity is markedly decreased by this lesion. These results were interpreted to indicate that at least a portion of D-3 binding identifies an autoreceptor on nigrostriatal terminals (Sokoloff et al., Nature 288:283, 1980; Nagy et al., Nature 274:278, 1978). However, in all of these studies no controls for the depletion of dopamine produced by this lesion were conducted. We have extended these studies and have observed the following: 1) Chronic 6-hydroxydopamine lesions produce increases in D-2 specific binding in striatum; 2) 6-Hydroxydopamine lesions or acute reserpine treatment (after Bacopolous, Life Sciences 29:2407, 1981) produces a marked (~50%) decrease in D-3 specific binding in striatum; and 3) decreases in D-3 binding produced by acute reserpine can be partially reversed by pre-incubating membranes in the presence of added dopamine or the first supernatants of control striatal homogenates. These findings question the proposal that D-3 binding identifies terminal autoreceptors and, along with other data to be presented, suggests that these binding sites are postsynaptic. Current studies are being conducted to determine how much, if any, of this binding represents labeling of a high affinity agonist binding state of the D-1 dopamine receptor. Supported by PHS MH32990.

208.4 IMMUNOCYTOCHEMICAL LOCALIZATION OF D-2 BINDING SITES IN THE RODENT FOREBRAIN.

A.C. Church, J.E. Kleinman* and R.J. Wyatt. Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Our understanding of the neurochemical circuitry of dopaminergic brain regions has been inhibited by an inability to precisely determine which cellular elements are postsynaptic to the the dopamine terminals. These postsynaptic sites should contain at least one of the different types of dopaminergic receptors described recently by Kebabian and Calne (1979). One of the dopamine receptors, the D-2 receptor, is potently inhibited by the drugs used to treat schizophrenia. A description of D-2 circuitry could lead to a better understanding of the schizophrenic syndrome. Although autoradiographic techniques provide histochemical quantification, they lack sufficient resolution. Therefore an attempt has been made to see the D-2 receptor site using immunocytochemical techniques.

Lisuride, a high affinity agonist for the D-2 receptor (K_D=5 x 10^{-10}M), was chemically linked to bovine serum albumin to form a conjugate. The lisuride-BSA conjugate was injected subcutaneously into rabbits and serums were screened for antibodies to both the conjugate and to lisuride. In order to see the lisuride binding sites, the anti-lisuride serum was then used in combination with the peroxidase anti-peroxidase unlabeled antibody technique.

Results indicate that of a high number of D-2 like binding sites are present in the mouse caudate nucleus and olfactory tubercle. Pericellular and apparent terminal staining was found in both brain areas and this staining was abolished by conincubation of tissues slices with both lisuride and excess fluphenazine. Further results on the localization of this apparent D-2 binding sites will be presented.

208.5 CHARACTERISTICS OF MATURING DA RECEPTORS AFTER IN UTERO EXPOSURE TO NEUROLEPTICS. Helen Rosengarten* and Arnold J. Friedhoff. Millhauser Lab., N.Y.U. Medical Center, New York, N.Y. 10016.

In a previous study we demonstrated an apparent down regulation of caudate DA receptors in pups following exposure to haloperidol during the entire gestational period (Science 203, 1133, 1979). We have now determined that haloperidol (1.25 mg/kg or 2.5 mg/kg) and cis-flupenthixol (2mg/kg) can alter the receptor number if administered to pregnant dams only during days 14-17, a period marked by dopaminergic neuronal differentiation and synaptogenesis. Scatchard analysis (^{3}H-spiroperidol binding) revealed a decrement of approximately 25% (Control B_{max} = 97 ± 0.06 fmoles /10 mg wet weight) in 15 day old pups, while Kd values remained unchanged (0.165 ± 0.5nM and 0.18 ± 0.02 nM for control and experimental pups, respectively).

There were no changes in Bmax or Kd in the mesolimbic region. cis-Flupenthixol (2 mg/kg) induced a similar change in caudate and failed to alter binding in the mesolimbic area.

Tissue level of haloperidol in 15 day old pups, treated in the prenatal period, measured by radioimmunoassay (Wurzburger et al. JPET 217,757, 1981) were found to be below the sensitivity of the procedure (20 pg/8 mg of caudate tissue). The addition of 20 pg of haloperidol to naive caudate membrane homogenates had no effect on ^{3}H-spiroperidol binding.

Implications of these findings for the role of dopamine in regulation of behavior and for human offspring exposed to neuroleptics in utero will be discussed.

208.6 POSSIBLE MODULATION OF STRIATAL D_2 DOPAMINE RECEPTORS BY SEROTONERGIC NEURONS. Daiga M. Helmeste and Siu Wa Tang. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada M5T 1R8.

Increased densities of D_2 dopamine receptors have been widely reported to exist in post-mortem schizophrenic brains (1). We were interested in investigating whether manipulation of the CNS serotonergic system could result in secondary changes in striatal D_2 dopamine receptor densities, as measured with ^{3}H-spiperone.

In the first experiment, rats were treated chronically with D-fenfluramine (serotonin releaser and uptake inhibitor), 2.5 mg/kg i.p., twice daily for 28 days. ^{3}H-Spiperone scatchard analysis (0.05 to 2.0 nM), using 10 µM sulpiride to define specific binding to D_2 receptors, showed a 20% decrease in receptor density in fenfluramine-treated rats (mean ± SE: 213 ± 8 fmoles/mg protein in controls, n = 5; 174 ± 7 in fenfluramine-treated, n = 5; P < 0.01) with no change in Kd (mean ± SE: 0.07 ± 0.01 nM for controls; 0.06 ± 0.01 for fenfluramine-treated).

In the second experiment, rats received electrolytic lesions of the raphe nuclei. Six weeks post-operatively, striatal homogenates were prepared and assayed for D_2 receptor binding using 0.2 nM ^{3}H-spiperone and 10 µM sulpiride to define specific binding. Raphe-lesioned rats which had approximately 80% or greater depletion of serotonin content showed a trend towards an increase in D_2 binding (mean ± SE: 147 ± 8 fmoles/mg protein, n = 6) of 15%, which was not statistically significant compared to controls (129 ± 8, n = 13). Raphe lesioned rats which had serotonin depletions of less than 80%, showed no such trend (121 ± 8, n = 6).

These results suggest that manipulation of the CNS serotonin system may have secondary effects on the dopamine system, as measured by D_2 receptor binding in the striatum. They also raise the possibility that the changes in D_2 receptor densities seen in post-mortem schizophrenic brains could result from changes in other neurotransmitter systems, such as the serotonergic system.

(1) Lee and Seeman, Soc. Neurosci. Abstr. 4: 496, 1978. Seeman, Pharmacol. Rev. 32: 229 (1980).

(We thank Dr. D. Coscina and Anne Dumbrille-Ross for providing the raphe-lesioned animals).

208.7 ^{3}H-S-SULPIRIDE, BUT NOT ^{3}H-SPIPERONE, LABELS A SINGLE DOPAMINERGIC BINDING SITE IN STRIATUM AND RETINA. Nancy R. Zahniser and Margarita L. Dubocovich. Dept. Pharmacol., Univ. Co. Sch.Med., Denver, CO 80262.

Functional studies have shown that both sulpiride and spiperone are antagonists at dopamine receptors of the D-2 subtype. We have compared the number and characteristics of the sites labeled by ^{3}H-S-sulpiride (New England Nuclear) with those labeled by ^{3}H-spiperone in order to determine the selectivity of each radioligand in binding to this dopamine receptor subtype.

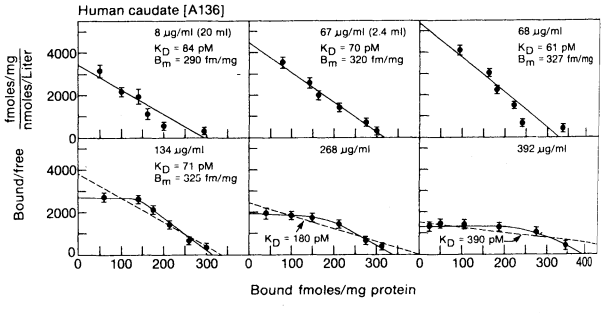
Binding to plasma membranes prepared from albino rabbit striatum and retina and rat striatum was carried out in Krebs' buffer (no ascorbate) at 37°. Samples were incubated for 20 min, and the reaction was terminated by dilution with ice-cold buffer and rapid filtration. Protein concentrations for ^{3}H-S-sulpiride assays were 0.20 mg for rabbit striatum, 0.30 mg for rabbit retina and 0.15 mg for rat striatum; these concentrations were 3-fold higher then those used for ^{3}H-spiperone assays. The relative potencies of drugs in inhibiting the binding of both radioligands in striatum were similar (spiperone = (+)butaclamol = domperidone = α-flupenthixol > S-sulpiride > N,N-di-n-propyldopamine > dopamine = β-flupenthixol > (-)butaclamol = R-sulpiride). Hill coefficients (n_H) for the inhibition of ^{3}H-S-sulpiride binding by both agonists and antagonists were not different from 1. In contrast, inhibition of ^{3}H-spiperone binding by agonists and most antagonists had n_H < 1. The notable exception was S-sulpiride for which n_H = 1. Scatchard analysis of ^{3}H-S-sulpiride binding was carried out only in striatal tissue. In striatum the number of sites labeled by ^{3}H-S-sulpiride as defined by spiperone and those labeled by ^{3}H-spiperone as defined by the isomers of sulpiride were not significantly different (rabbit: 412 + 87 fmol/mg protein vs. 537 + 36 and rat: 376 + 48 vs. 336 + 22; N = 3-6). Similar results were obtained when isomers of butaclamol were used to define specific binding. Using the isomers of flupenthixol to define the specific binding of ^{3}H-spiperone in rabbit striatum and retina, significantly more sites were labeled (135% in striatum and 160% in retina) than when specific binding was defined with the isomers of sulpiride. In contrast, the number of striatal ^{3}H-S-sulpiride binding sites were identical whether spiperone or the isomers of flupenthixol were used to define specific binding. The results indicate that ^{3}H-S-sulpiride labels a single site with an affinity of approximately 10 nM regardless of how specific binding is defined. ^{3}H-Spiperone appears to label the same site if specific binding is defined with the isomers of sulpiride but labels additional sites when specific binding is defined with the isomers of flupenthixol. It is most likely that the single site labeled by ^{3}H-S-sulpiride is the D-2 receptor subtype. (Supported by USPHS NS 09199 and BRSG 05357)

208.8 DOPAMINE RECEPTOR PARAMETERS (DETECTED BY ^{3}H-SPIPERONE) DEPEND ON TISSUE CONCENTRATION. P. Seeman, C. Ulpian* and J. Wells*. Pharmacology Dept., Faculties of Medicine and Pharmacy, University of Toronto, Toronto, Canada M5S 1A8.

Since a very wide range of K_D values (13 pM to 1600 pM) had been reported in the literature for ^{3}H-spiperone binding to dopamine receptors, we investigated whether the apparent K_D could vary with the final concentration of membrane protein in the incubate.

As shown in the Fig., we found that below a concentration of 100 µg protein per ml of final incubate, the Scatchard relation was linear for the binding of ^{3}H-spiperone to (previously frozen) human striatum, yielding K_D values of about 50 pM. At concentrations of protein higher than 100 µg per ml incubate, both the K_D and B_{max} (density of binding sites) increased significantly, if one assumed that the Scatchard relation was still linear. As shown in the Fig., however, such high concentrations of protein did not yield a linear Scatchard relation. The nonlinearity stems from the depletion of the fat-soluble ^{3}H-spiperone to the membrane.

We conclude that the K_D and B_{max} values for ^{3}H-spiperone are only reliably measured at concentrations of protein less than 100 µg per ml incubate. We also found that repeated washing of the tissue removed 10 to 58% (average 20%) of the receptors, but 30% of the protein; we thus recommend that the tissue not be washed when measuring the total density of dopamine receptors per gram of wet tissue, but that the tissue be simply homogenized and finally diluted to between 50 and 100 µg protein per ml incubate.

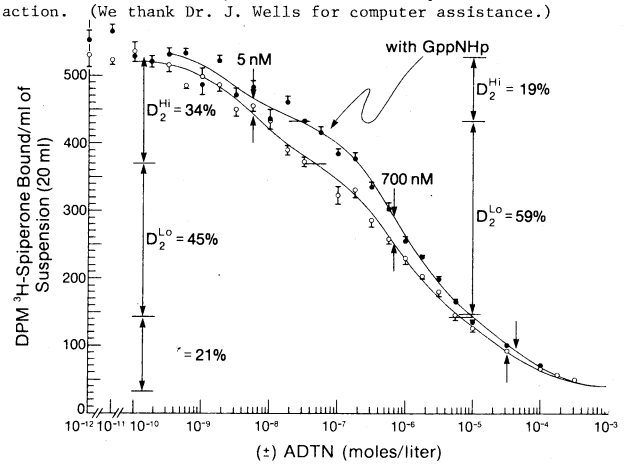


208.9 MODULATION OF THE D_2 DOPAMINE RECEPTOR IN CALF CAUDATE BY GUANINE NUCLEOTIDE. K.A. WREGGETT*, S.R. GEORGE* and P. SEEMAN. (SPON: J. Khanna). Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

^{3}H-Neuroleptics can label dopaminergic sites with differing affinities for agonists. Agonist/^{3}H-spiperone (^{3}H-SPIP) curves have low Hill slopes and are sensitive to guanine nucleotides. We quantitatively tested whether the nucleotide could totally convert ^{3}H-SPIP sites with high affinity for the agonist (D_2^{Hi}) into sites with low affinity for the agonist (D_2^{Lo}). Total conversion was not found.

To minimize depletion of the radioligand a final volume of 20 ml with 40 µg protein/ml was used. The competition of ADTN versus 50 pM ^{3}H-SPIP was analysed by computer. The shallow curve (n_H = 0.45) was resolved into three sites discriminated by ADTN. With 100 µM Gpp(NH)p the curve steepened slightly (n_H = 0.49); the high-affinity (5 nM) component (D_2^{Hi}) decreased from 34% to 19% with an increase in the low-affinity (700 nM) component (D_2^{Lo}) from 45% to 59%. The very-low-affinity (50 µM) site was not affected (21% of total sites).

The results indicate that guanine nucleotides can convert about half of the D_2^{Hi} sites into D_2^{Lo} sites. Such incomplete conversion suggests that the remaining D_2^{Hi} sites are either distinctly different sites or else may arise from the receptor-nucleotide interaction. (We thank Dr. J. Wells for computer assistance.)



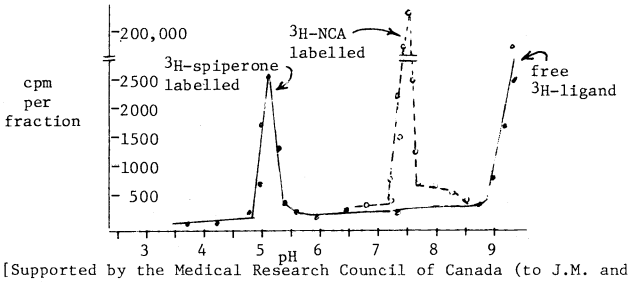
208.10 IRREVERSIBLE ^{3}H-LIGANDS FOR DOPAMINE RECEPTOR PURIFICATION. L. Lilly*, J. Magnan*, A. Davis and P. Seeman. (SPON: S.W. Tang). Departments of Pharmacology, University of Toronto, and Psychopharmacology, Clarke Institute of Psychiatry, Toronto, Canada.

Established protein purification techniques can be readily applied to receptor isolation if a specific, irreversible, ^{3}H-ligand is available. We report the use of two ligands, ^{3}H-phenoxybenzamine (POB) and ^{3}H-(-)-chloroethylapomorphine (NCA; gift of New England Nuclear, Boston) in purifying dopamine sites from mammalian brain.

^{3}H-POB, at a concentration of 10 nM, completely associated with digitonin-solubilized canine striatum after 90 minutes. Less than 20% of the binding, however, was found to be dopamine (D_2) related as defined using 10 µM (+)-butaclamol; the remainder was to alpha- and beta-adrenoceptors, among others. Thus, the lack of specificity proved to be a disadvantage for ^{3}H-POB.

It has been suggested by Baldessarini et. al. (Eur. J. Pharm. 67: 105-110, 1980) that NCA might prove to be a useful ligand in dopamine receptor isolation and characterization. To investigate if ^{3}H-NCA could label an agonist conformation of the dopamine receptor, we compared it with the D_2 receptor as labelled by ^{3}H-spiperone. On striatal membranes, NCA was not a potent displacer of ^{3}H-spiperone binding. In the solubilized preparation, incubation of 10 nM ^{3}H-NCA for 90 minutes was found to label a site of the same isoelectric point (pI 7.5) and molecular size as did ^{3}H-dopamine (4 nM, 16 hour incubation). However, ^{3}H-spiperone-labelled D_2 receptors have an isoelectric value of pH 5.06. (See Fig.) ^{3}H-NCA does not, therefore, appear to label the ^{3}H-spiperone-binding protein. We are currently studying the cross-reactivity of D_2 receptor antibodies with the ^{3}H-NCA-labeled site

Fig. Isoelectric focusing of solubilized canine striatum.



[Supported by the Medical Research Council of Canada (to J.M. and P.S.), C.K. Clarke and Clarke Associates (to A.D.) and Ontario Government Scholarship (to L.L.)].

208.11 MANGANESE AND DOPAMINE RECEPTORS (D-2 TYPE). B.K. Madras and B. Chan* Psychopharmacology Section, Clarke Institute of Psychiatry, Toronto, Ontario, Canada. M5T 1R8.

There is increasing recognition that the membrane matrix may influence the stability, affinity, and binding capacity of membrane receptors. Ascorbic acid, manganese, and EDTA modulated ^{3}H-spiperone binding to membrane-but not solubilized dopamine receptors (Madras et al 1981, Chan et al, 1982). Ascorbate produced a profound time-dependent decrease in ^{3}H-spiperone binding to membranes which was prevented by EDTA or manganese (Mn^{++}). Ascorbate and Fe^{++}-catalyzed lipid peroxidation of unsaturated fatty acids in the membranes was proposed as the mechanism.

In the present study the effect of manganese on striatal dopamine receptors in vitro and in vivo is reported. In vitro, membranes were preincubated with various buffers, washed, and then assayed for ^{3}H-spiperone binding. Manganese modulation of preincubated dopamine receptors was a function of buffer constituents. Using TEAN buffer (Tris-Na_2 EDTA-Ascorbate-Nialamide), Mn^{++} had no effect on (+)-butaclamol or dopamine displacement of ^{3}H-spiperone binding. In TN buffer (Tris-Nialamide), Mn^{++} enchanced dopamine displacement of ^{3}H-spiperone binding slightly, but was without effect on the (+)-butaclamol displacement curve. Preincubation in TAN buffer (Tris-Ascorbate-Nialamide) in the absence of Mn^{++} markedly reduced total and specific ^{3}H-spiperone binding. The displacement curves for both (+)-butaclamol and dopamine were shifted to the right, increasing the $I.C._{50}$ values for (+)-butaclamol (8-fold) and dopamine (2-fold). Mn^{++} added to the preincubation buffer reversed the effects of ascorbate. Thus Mn^{++} modulation of dopamine receptors occurs mainly in the presence of ascorbic acid. Mn^{++} inhibition of lipid peroxidation (Shukla and Chandra, 1981) may account for these observations.

Administration of Mn^{++} to rats increased dopamine receptor density by 10% after 1 week and by 17% after 2 weeks. Whether the increase in receptor number is related to Mn^{++} inhibition of lipid peroxidation is not known. An increase in dopamine receptor density may also occur in manganese-induced psychosis. The physiological relevance of manganese modulation is not known.

208.12 ALLOSTERIC REGULATION OF 3H-DOPAMINE BINDING BY GUANYL NUCLEOTIDES: SIMILARITIES WITH THE INHIBITORY MECHANISM OF ANTIPSYCHOTIC DRUGS. N.G. Bacopoulos (SPON: R. Rosenstein) Departments of Pharmacology and Psychiatry, Dartmouth Medical School, Hanover, New Hampshire 03755.

We have previously reported (Biochem. Pharmacol. 30: 2037,1981; Life Sciences, 29: 2407, 1981) that preincubation of homogenates of rat caudate nucleus at 37° C quadrupled the stereospecific binding of 3H-dopamine (3H-DA) to the subsequently washed membranes. We now report that the addition of the guanyl nucleotides guanosine triphosphate (GTP), guanosine diphosphate (GDP) or the nonhydrolyzable analogue guanylyl imidodiphosphate (GPP(NH)P) to the preincubation reduced the number of 3H-DA binding sites (Bmax) from 0.6 to 0.3 pmoles/mg protein, without changing the equilibrium dissociation constant (Kd) which remained 1.5 nM. The addition of antipsychotic drugs (APD) to the preincubation also lowered 3H-DA binding by a noncompetitive mechanism. APD were noncompetitive inhibitors of 3H-DA binding when added directly to the binding assay which was carried out at 23° C. In contrast guanyl nucleotides were very weak inhibitors of 3H-DA binding when added at this step.

When both APD and nucleotides were added to the preincubation the inhibition of 3H-DA binding was additive suggesting that two different mechanisms of noncompetitive inhibition were involved. In the presence of 0.5 mM GDP, for example, 50 nM fluphenazine lowered 3H-DA binding by 83%; 100 nM chlorpromazine by 74% and 100 nM haloperidol by 70%. In the absence of nucleotide inhibition was 45, 28 and 35% with each APD. The potentiation was significant (p less that 0.01 by group t-test) in each case.

Because the APD-induced inhibition is noncompetitive, it will occur in spite of the higher relative affinity of dopamine for its high-affinity binding sites, if drug concentrations near the Ki are reached. The above results suggest that the concentrations of APD occurring in the plasma of schizophrenic patients (Cohen et al., Psychiatr. Res. 1: 173, 1980) are sufficient to induce at least a partial inhibition of the association of dopamine to its high affinity receptors if similar drug concentrations occur in the brain. The in vivo pharmacologic and clinical effects of APD may therefore represent an integration of their actions on the receptors discussed here and their actions on the previously described "D-2" receptors labeled with high affinity by 3H-butyrophenones. Supported by USPHS research grant MH33958.

208.PO DOPAMINERGIC BINDING SITES IN THE ANTERIOR PITUITARY DISCRIMINATED BY AGONISTS AND GUANINE NUCLEOTIDES. S.R. GEORGE*, K.A. WREGGETT* and P. SEEMAN. (SPON: Y. Israel). Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

The bovine anterior pituitary (AP) gland contains dopamine (DA) receptors that can be labeled by ^{3}H-neuroleptics. We examined whether ^{3}H-spiperone(spip) labels one or more populations of binding sites displaceable by a dopamine agonist, ADTN ([±]-6,7-dihydroxy-2-aminotetralin) and whether such populations might be totally interconvertible. To minimize the depletion of radioligand, we used 40-50 µg protein/ml, 50-90 pM spip and large incubation volumes (20 ml). Specific spip binding was defined using 500 nM haloperidol and represented 70-80% of total binding. Antagonist competitions of bound spip were monophasic and steep (n_H=1), indicating a DA binding site(s) with uniform affinity for antagonists. In contrast, agonist competitions were shallow (n_H<1), indicative of more than one site discriminated by agonists. These data could be resolved by computer modeling into two sites with high and low affinities for agonists. The rank order of agonist potencies was n-propylnorapomorphine (NPA) > ADTN > apomorphine(APO) > DA for the high-affinity site, and NPA > APO > ADTN > DA for the low-affinity site.

The DA receptor in the AP inhibits adenylate cyclase, and guanine nucleotides modulate DA-sensitive adenylate cyclase. We examined, therefore, the effects of a nonhydrolyzable GTP analogue, GppNHp, on the agonist differentiated sites:

		CONTROL	WITH 100 µM GppNHp
Hill slope for ADTN, n_H		0.61 ± 0.05	0.84 ± 0.04
HIGH AFFINITY FOR ADTN	K(nM)	4.8 ± 1.0	1.3 ± 0.1
	% sites	18.4 ± 1.0	8.1 ± 2.1
LOW AFFINITY FOR ADTN	K(nM)	2369 ± 395	2090 ± 218
	% sites	81.6 ± 2.2	90.8 ± 3.3

In summary, it appears the DA binding sites of AP have uniform affinity for antagonists, but differential high and low affinities for agonists. GppNHp induced a shift in about half of the sites with high affinity for ADTN (18.4%) to sites with low affinity for ADTN. The nature and significance of the remaining GppNHp-insensitive, high-affinity, agonist-discriminated-DA-site (8.1%) remains to be shown.

(We thank Dr. J. Wells for computer assistance. Supported by the Medical Research Council of Canada.)

209.1 SHORT TIME SCALE INTERACTIONS BETWEEN BRAIN STEM NEURONS WITH SYMPATHETIC NERVE-RELATED ACTIVITY. S. M. Barman, S. F. Morrison and G. L. Gebber. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

The 2-6 c/s rhythm (normally cardiac-related) in sympathetic nerve discharge (SND) is believed to be representative of the fundamental organization of the brain stem sympathetic generator. In an attempt to define the mechanisms responsible for this rhythm, we have begun an examination of the short time scale (milliseconds) interactions between closely adjacent brain stem neurons with spontaneous activity related to inferior cardiac SND. Experiments were performed on baroreceptor-innervated and -denervated cats. Spike-triggered averaging was used to locate brain stem neurons with sympathetic nerve-related activity. Crosscorrelation analysis was performed in instances when it was possible with window discrimination to separate the spikes of two neurons located in the recording field of a single microelectrode. Unit pairs with sympathetic nerve-related activity were located in the medullary reticular formation and caudal raphe nuclei.

The discharges of 13 of 21 pairs of reticular neurons and 7 of 28 pairs of raphe neurons were correlated on a time scale of several ms. The discharges of the neurons in each pair were synchronized to the rising phase of the 2-6 c/s sympathetic nerve slow wave. Three patterns of short time scale interaction were observed both in baroreceptor-innervated and -denervated cats. The unit 1 $\rightarrow$ unit 2 crosscorrelogram for 11 neuronal pairs contained a sharp paracentral peak 1-4 ms either to the right or left of zero lag. Zero lag denotes the spike occurrence of unit 1. A paracentral peak may be indicative of a direct connection (mono-or oligosynaptic) between the two neurons whose discharges were recorded. Alternatively, the neurons may have shared input from a common source over pathways with slightly different conduction times. The crosscorrelograms for two neuronal pairs contained a peak both to the left and right of zero lag. Such peaks were located 1-5 ms from zero lag. This type of relationship may be indicative of reciprocal excitatory connections between the two neurons. The crosscorrelograms for 7 neuronal pairs exhibited a dispersed pericentral peak (i.e., around zero lag). A pericentral peak most likely results from input shared by the two neurons under study. These results suggest that direct interconnection and/or shared input account for synchronization of the discharges of elements of the brain stem network responsible for the rising (i.e., excitatory) phase of the 2-6 c/s sympathetic nerve slow wave.

The high incidence (62%) of short time scale interactions among reticular neurons with sympathetic nerve-related activity is in marked contrast to the low incidence (25%) of same among raphe neurons. The difference was statistically significant ($p=0.01$; Fisher's exact test). This result suggests fundamental differences in the organization of sympathetic networks located in the two regions. (Supported by PHS Grants HL-13187, NS-06693 and a Michigan Heart Association Grant-in-Aid.)

209.2 EVIDENCE FOR A RECURRENT INHIBITORY LOOP IN THE BRAIN STEM SYMPATHETIC NETWORK. S. F. Morrison, S. M. Barman and G. L. Gebber. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

Spike-triggered averaging was used to identify single neurons in the caudal raphe nuclei and medullary reticular formation of the cat with spontaneous activity related to inferior cardiac sympathetic nerve discharge (SND). Two types of neurons with activity synchronized to the rising (i.e., excitatory) phase of the cardiac-related slow wave of SND were found in each region. Type 1 units were inhibited during 5 s periods of elevated carotid sinus pressure while type 2 units were excited by baroreceptor reflex activation. The two unit types responded identically to other inputs. Single shock activation of posterior hypothalamic sites or sciatic nerve afferents excited both unit types and elicited a negative potential in the inferior cardiac nerve. The interval between electrically evoked unit and sympathetic nerve responses was equivalent to that between spontaneously occurring type 1 or type 2 unit discharge and the peak of the cardiac-related sympathetic nerve slow wave. In addition, unit and inferior cardiac nerve responses elicited by hypothalamic or sciatic nerve stimulation were followed by equivalent periods of reduced background discharge. Finally, the discharges of both unit types remained locked in time to the peak of the cardiac-related slow wave in SND when the heart period was changed by ventricular pacing.

We propose the following model to explain our results. First, type 1 units are elements of the brain stem network responsible for the excitatory phase of the sympathetic nerve slow wave since they were inhibited by baroreceptor reflex activation and excited by inputs which increase SND. Type 2 units were excited both by inputs which inhibit or excite type 1 units. These observations raise the possibility that type 2 units supply feedforward (via baroreceptors) and feedback (i.e., recurrent) inhibition to elements of the brain stem generator (i.e., type 1 units) responsible for the excitatory phase of the sympathetic nerve slow wave.

Additional observations are consistent with the possibility that feedforward and recurrent inhibition mediated through the type 2 neuron are responsible for termination of the excitatory phase of the sympathetic nerve slow wave. First, the amplitude and duration of the rising phase of the sympathetic nerve slow wave were increased when ventricular pacing was used to delay the onset of pulse synchronous baroreceptor nerve discharge. This observation indicates that termination of the excitatory phase of SND can be advanced by baroreceptor nerve discharge. Second, termination of the excitatory phase of SND occurred before the onset of pulse synchronous baroreceptor nerve discharge at heart rates below 2 beats/s. The proposed recurrent inhibitory loop may be responsible for SND cutoff under these conditions. (Supported by PHS Grants HL-13187, NS-06693 and a Michigan Heart Association Grant-in-Aid.)

209.3 HYPOTHESIZED FACILITATION OF DIGITALIS-INDUCED CARDIOTOXICITY THROUGH THE AREA POSTREMA IS DISPROVED. Herbert L. Borison, C. Dennis Thron* and José A. Riancho*. Institute of Brain Stem Studies, Dartmouth Medical School, Hanover, NH 03755.

The area postrema (AP) is recognized as the morphologic locus of the emetic chemoreceptor trigger zone (CTZ) and the site of digitalis-induced vomiting. A central excitatory factor in digitalis-induced cardiotoxicity is suspected because acute high spinal cord section in cats afforded a protective effect (Somberg, Risler and Smith, Am. J. Physiol. 1978, 235:H531). Since no protection was obtained after section of the medulla oblongata two mm above the obex, Somberg and Smith (Science, 1979, 204:321) implicated AP as the likely site of the neural excitation.

Our experimental conditions for testing digitalis cardiotoxicity were as follows. Male cats, anesthetized with pentobarbital (40 mg/kg i.p.), were prepared for recording blood pressure, heart rate, ECG (lead II and sup. vena cava), and end-expiratory pCO_2 (maintained ca. 35 mm Hg). One hour after bilateral vagotomy, an infusion of ouabain (a digitalis glycoside) was started at 1.0 µg/kg/min intravenously. ^{3}H-ouabain was incorporated as a tracer for assay of drug content in heart muscle. The experiment was terminated when "ventricular tachycardia" appeared as four consecutive beats. The heart ventricles were biopsied promptly for liquid scintillation counting.

We performed three groups of experiments to evaluate the hypothesized facilitatory role of AP as well as the primary claim for a central neuroexcitatory factor. (Group 1) AP was ablated in 4 cats, including two chronic preparations demonstrated to be unresponsive to the emetic action of digitalis and verified histologically. (group 2) Sham exposure of AP was made in 10 cats, including two chronic preparations that vomited in response to digitalis. (Group 3) High spinal cord section (plus sham exposure of AP) was made in 7 cats. The amount of ouabain infused to produce ventricular tachycardia varied from 37 to 87 µg/kg for the entire series. Grouped dose values (mean ± S.D. in µg/kg) were respectively: (1) 62.3 ± 17.39; (2) 57.3 ± 10.8; (3) 66.9 ± 16.12. With regard to the cardiac content of ouabain at the onset of the arrhythmia, grouped levels (mean ± S.D. in µg/g) were respectively: (1) 0.53 ± .101; (2) 0.56 ± .090; (3) 0.58 ± .073. No statistically significant difference was demonstrable between groups by any cross comparison either in the infused amount of ouabain or in the ouabain content of the heart.

Thus, our results invalidate the hypothesis of digitalis-induced cardiotoxicity facilitated through the area postrema. Moreover, we were unable to demonstrate a protective effect of high spinal cord section in the arrhythmogenic drug action. (Supported by the New Hampshire Heart Association)

209.4 DIRECT PATHWAY FROM CARDIOVASCULAR NEURONS IN THE VENTROLATERAL MEDULLA TO THE PARAVENTRICULAR NUCLEUS IN THE CAT. J. Ciriello, M. M. Caverson* and T. X. Zhang* (SPON: J. P. Girvin). Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

Physiological and pharmacological studies have implicated the ventrolateral medulla (VLM) and the paraventricular nucleus (PVH) in the central cardiovascular regulation and in the release of vasopressin. Although these findings suggest that the VLM can influence the activity of neurons in the PVH, this interpretation is not unequivocal. The present study was done to provide anatomical and electrophysiological evidence for a direct pathway carrying cardiovascular afferent information between these two structures in the cat. In the first series of experiments horseradish peroxidase (HRP) was allowed to diffuse from glass micropipettes (50-100 µm, internal diameter) into the regions of the PVH. After a survival period of 2-5 days, brain stem sections were processed according to the tetramethyl benzidine method. Labelled cells were found bilaterally in the VLM primarily in an area just dorsal and lateral to the lateral reticular nucleus, which extended from the level of the decussation of the pyramids to the level of the caudal pole of the facial nucleus. In addition, a small collection of labelled cells was found predominantly on the contralateral side, scattered along the ventral surface of the brain stem. In the second series of experiments areas of the VLM containing labelled cells were explored for single units antidromically activated by stimulation of the PVH in chloralosed, paralyzed and artificially ventilated cats. Identified units were further tested for their response to stimulation of the carotid sinus (CSN) and aortic depressor (ADN) nerves. Of 23 antidromically identified units which were excited with latencies in the range of 2.1 - 14 ms, 15 units responded orthodromically by excitation only to stimulation of the CSN (latency range, 2.5 - 15 ms), and 4 units were excited orthodromically to both buffer nerves (latency range, 4 - 12 ms and 8 - 18 ms for CSN and ADN, respectively). The remaining 4 units did not respond to stimulation of either nerve. These results provide anatomical and electrophysiological evidence of a monosynaptic cardiovascular pathway connecting the VLM and the PVH, and suggest that this ascending pathway is involved in the release of vasopressin by PVH neurons during activation of baro- and chemoreceptor afferent fibers.

(Supported by the Ontario Heart Foundation)

209.5 NEURONS IN THE VENTROLATERAL MEDULLA PROJECTING DIRECTLY TO THORACIC SPINAL SYMPATHETIC AREAS RECEIVE CARDIOVASCULAR AFFERENT INPUTS. M. M. Caverson*, J. Ciriello and F. R. Calaresu (SPON: V. Hachinski). Department of Physiology, The University of Western Ontario, London, Canada, N6A 5C1.

The reticular formation in the ventrolateral medulla has received considerable attention recently as an important integrating center in the control of arterial pressure. In a preliminary anatomical study with the horseradish peroxidase method we have demonstrated efferent connections from neurons in specific regions of the ventrolateral medulla to sympathetic areas within the thoracic cord. In the present study we have attempted to characterize these neurons in the ventrolateral medulla by investigating their responsiveness to stimulation of cardiovascular afferent inputs. Experiments were done in chloralosed, paralyzed and artificially ventilated cats. Single units were identified in the reticular formation of the ventrolateral medulla by antidromic excitation to stimulation of functionally and histologically verified sites in the region of the intermediolateral cell column at the level of T2. These antidromically identified neurons were studied for their responses to stimulation of the carotid sinus and aortic depressor nerves, and of pressor sites in the paraventricular nucleus of the hypothalamus. Twenty units were antidromically excited in the region of the ventrolateral medulla. The latency to stimulation in the thoracic cord ranged from 0.8 to 6.8 ms, corresponding to conduction velocities of 15 to 125 m/s. Of these 20 units 12 were orthodromically excited by stimulation of only the CSN (latency range 4.2 - 36 ms), three were excited only by stimulation of the aortic depressor nerve (latency range, 4.4 to 25 ms), and two units were activated by stimulation of both buffer nerves. In addition, two units responded to stimulation of only the paraventricular nucleus and one was excited by all three inputs. These data provide electrophysiological evidence of direct efferent connections from neurons within the ventrolateral medulla which relay cardiovascular afferent information from the buffer nerves and hypothalamus to spinal sympathetic areas in the thoracic cord.

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209.6 ELECTRICAL ACTIVITY IN RENAL NERVES EVOKED BY STIMULATION OF CERVICAL VAGUS IN THE CAT. A. Stella*, J. Ciriello and F. R. Calaresu. Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

It is widely believed that the nerve supply to the mammalian kidney is primarily of sympathetic origin although in a recent anatomical study we have demonstrated that neurons in the dorsal motor nucleus of the vagus are labelled after injection of HRP into the kidney. In this study we have attempted to establish electrophysiologically the existence of connections between the cervical vagus and renal nerves in the cat by recording from the central segment of cut renal nerves during electrical stimulation of the cervical vagus. Experiments were done in cats anaesthetized with alphachloralose, paralyzed and artificially ventilated. Arterial pressure and heart rate were continuously monitored on a polygraph. Branches of the left renal nerves were dissected retroperitoneally and were cut just proximal to their entry into the renal hilum. The cervical vagi were exposed and separated from the aortic depressor nerves. Stimulating bipolar stainless steel electrodes were placed just distal to the nodose ganglion and the vagi were crushed proximal to the electrodes. Stainless steel bipolar recording electrodes were used to record electrical activity from the central segments of the renal nerves. To eliminate changes in cardiovascular variables during stimulation of the cervical vagi atropine methylbromide was administered intravenously after determining the threshold current for eliciting cardiac slowing. Single pulse electrical stimulation of the peripheral segment of the contralateral cervical vagus at 3 times the threshold current for bradycardia consistently elicited compound action potentials in the renal nerves. Three different components were observed: an early response with a mean peak latency of 54 $\pm$ 5 ms, a response with a mean peak latency of 130 $\pm$ 15 ms, and a late response with a mean peak latency of 494 $\pm$ 15 ms. An additional response with a 175 $\pm$ 5 ms latency was observed in some cases. Electrical stimulation of the ipsilateral cervical vagus elicited similar responses with mean peak latencies of 52 $\pm$ 5, 116 $\pm$ 10 and 503 $\pm$ 12 ms, respectively. An additional component with a mean peak latency of 184 $\pm$ 3 ms was evoked in a few instances. No differences were observed in the potentials evoked by single pulse stimulation and those evoked by trains of 5 pulses at 200 Hz. Intravenous injection of hexamethonium bromide (10 mg/kg) abolished all the responses evoked by vagal stimulation. These experiments demonstrate the existence of fibers in the renal nerves of the cat which are activated by stimulation of preganglionic axons present in the cervical vagus and suggest that vagal efferent fibers play a physiological role in the regulation of renal function.

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209.7 IS THE TURNOVER OF NOREPINEPHRINE IN THE HEART A USEFUL INDEX OF GENERAL SYMPATHETIC ACTIVITY TO THE CARDIOVASCULAR SYSTEM? K. P. Patel* and R. L. Kline* (SPON: J. Ciriello). Dept. of Physiol., Univ. of Western Ontario, London, Ontario, CANADA N6A 5C1.

Many studies have used the turnover of norepinephrine (NE) in the heart as an index of general sympathetic activity to the cardiovascular system. In particular, this measurement has been used to provide evidence for an enhanced sympathetic activity in several models of experimental hypertension. In the present study we measured the turnover of NE in the left ventricle of the heart, kidney, and skeletal muscle in conscious Wistar rats by measuring the decline of [NE] 90 min after administration of α-methyl-p-tyrosine and subjecting the animals to acute hypotension or hypertension by continuous infusion of nitroprusside or phenylephrine, respectively, for 60 min. [NE] at 90 min was expressed as a percent of the initial value for each tissue and the difference between the experimental and control groups (saline infused normotensive rats) was assessed using a t-test.

Mean arterial pressure (measured by indwelling catheter in unrestrained rats) was maintained at least 25 mm Hg above or below the control mean pressure for each animal during the 60 min of hypotension or hypertension. Heart rate changed initially by over 100 beats/min in response to the two conditions, and remained increased by at least 56 beats/min throughout the hypotensive period, and decreased by at least 80 beats/min during hypertension. There was a significant increase in turnover of NE in all organs studied in the hypotensive group, as would be predicted by classical reflex mechanisms. In the hypertensive group there was no significant change in turnover of NE in the kidney and skeletal muscle, suggesting little sympathetic tone in these organs under resting conditions. However, the turnover of NE was significantly increased in the left ventricle of hypertensive rats, even though the arterial pressure was elevated. These results suggest that the turnover of NE in the heart is not a good index of the overall peripheral sympathetic activity. This observation is of significance considering that the turnover of NE in the heart is often used to suggest a cause and effect relationship between sympathetic nerve activity and hypertension.

(Supported by the Ontario Heart Foundation)

209.8 THE ROLE OF SPINAL CORD CATECHOLAMINES IN THE CARDIOVASCULAR RESPONSE TO ACTIVATION OF THE A5 CATECHOLAMINE CELL GROUP. J.J. Neil and A.D. Loewy. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

The A5 catecholamine cell group projects to the intermediolateral cell column of the spinal cord and to three medullary sites: the nucleus of the tractus solitarius, the dorsal motor nucleus of the vagus, and the paramedian reticular function (Brain Res. 174:309, 1979). Activation of cells in the A5 area of rats with L-glutamate leads to a dose-dependent decrease in heart rate and blood pressure. This effect is abolished by pretreating the rats with intraventricular 6-hydroxydopamine, which destroys catecholaminergic nerve terminals. This effect is also abolished in guanethidine-sympathectomized rats, which indicates that it is mediated by the sympathetic nervous system.

To determine if the A5-spinal projection is required for the decrease in blood pressure in response to activation of cells in the A5 area, animals were pretreated with bilateral injections of 2 µl 12 mg/ml 6-hydroxydopamine into the lower cervical spinal cord. Control studies using horseradish peroxidase and radiolabeled amino acid tracing techniques indicate that this treatment destroys the A5-spinal projection while leaving the A5-medullary projections intact. Four weeks following the spinal cord injection, rats were injected with L-glutamate into the region of the A5 cell group. In vehicle-treated control animals, the maximum decrease in systolic blood pressure following L-glutamate injection was 23±2 mm Hg (mean±SEM, n=7) versus 22±3 mm Hg (n=6) in the animals treated with intraspinal 6-hydroxydopamine.

These results suggest that the A5-medullary projections are capable of mediating the depressor response in the absence of the A5-spinal pathway.

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209.9 TONIC VASOMOTOR CONTROL BY A REGION OF ROSTRAL VENTROLATERAL MEDULLA CONTAINING C1 ADRENALINE NEURONS. C.A. Ross, D.A. Ruggiero and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

A region of the rostral ventrolateral medulla (RVL) contains neurons or fibers which tonically excite preganglionic vasomotor neurons (Dampney and Moon, Am. J. Physiol. 239, H349, 1980). Adrenaline neurons of the C1 group (Hokfelt et al., Brain Res. 66, 235, 1974) are also concentrated within part of the RVL. We sought to determine whether cardiovascular actions of the RVL could be attributed to C1 neurons and whether neurons in the RVL project directly to the thoracic intermediolateral column (ILC).

Electrical stimulation (100 Hz, 0.5 msec, 10 sec trains) of the RVL in chloralose (60 mg/kg) anesthetized, paralyzed, artificially ventilated rats elicited an elevation of arterial pressure (AP) and heart rate (HR). The threshold for a 10 mm Hg rise of AP was 9.5 $\pm$ 1.1 μA. At 5x threshold current, the increase of AP was 81.6 $\pm$ 2.5 mm and of HR was 73.3 $\pm$ 13.0 bpm (n=6). The medulla was systematically mapped in 10 rats with low intensity stimuli (25 μA) for pressor sites. Regions of RVL with the largest pressor responses corresponded to locations of C1 neurons labeled immunocytochemically with antibodies to the adrenaline synthesizing enzyme phenylethanolamine-N-methyltransferase (PNMT). Another pressor site in the dorsomedial medulla corresponded to the location of a bundle of PNMT-labeled efferent fibers.

Unilateral microinjection (n=12) of L-glutamate (50 pmoles to 10 nmoles in 100 nliters) into the RVL, but not adjacent regions, elicited dose-dependent increases in AP (to 70 mm). Thus, the RVL pressor response arises from excitation of perikarya and not axons. In contrast, unilateral injections (n=6) of GABA (100 pmoles to 10 nmoles) elicited dose-dependent decreases in AP (to 30 mm), while the GABA antagonist bicuculline (50 pmoles) caused prolonged (10-15 min) increases in AP (70 mm). Bilateral injection (n=6) of tetrodotoxin (10 pmoles) caused falls in AP to 51.7 $\pm$ 4.7 mm; subsequent cervical spinal transection caused no further fall (to 52.5 $\pm$ 6.2 mm).

In order to establish whether the RVL projects directly to the ILC, 20-50 nl of ^{3}H-leucine and ^{3}H-proline were injected into the pressor zone of the RVL of 8 rats anesthetized with halothane. Anterogradely transported label in the thoracic spinal cord was restricted to the ILC.

We conclude: 1) portions of the RVL containing C1 neurons give rise to a highly specific projection to the ILC; 2) neurons in the same area of RVL excite the sympathetic outflow; 3) these neurons are tonically active and appear solely responsible for sympathetic tone; 4) these neurons are also tonically inhibited, perhaps by GABAergic mechanisms.

We propose that the neurons in the RVL responsible for the maintenance of resting vasomotor tone are, at least in part, adrenaline neurons of the C1 group.

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209.10 MEDULLARY VISCERAL REFLEX ARCS DEMONSTRATED BY A COMBINED RETROGRADE AND ANTEROGRADE TRANSPORT TECHNIQUE. D.A. Ruggiero, C.A. Ross and D.J. Reis, Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

An area of rostral ventrolateral medulla (RVL) containing C1 adrenaline neurons innervates autonomic centers of spinal cord (Ross et al. Neurosci. Abst., 1982). Chemical or electrical stimulation of these neurons increases while their inactivation lowers arterial pressure. Close to the C1 cells in RVL are vagal motor neurons including those innervating the heart (Nosaka et al., J. Comp. Neurol., 1980). We sought to determine whether this area of RVL is innervated by neurons from portions of NTS integrating cardiovascular reflexes. To do this we have: 1) used retrograde transport of horseradish peroxidase (HRP), and 2) developed a new method combining retrograde transport of fast blue dye (FB) and anterograde transport of HRP in the same experiment.

After HRP injections restricted to the RVL, neurons were labeled in cardiovascular portions of the NTS, including its medial, interstitial, ventrolateral, and dorsal subdivisions. FB (2 μl of 2% solution in distilled water) was injected into the lower thoracic spinal cord (T9) or cervical vagus of rats anesthetized with halothane. Two to four days later 50 nl of a 1% solution of wheat germ agglutinin-HRP conjugate was injected into NTS. Two days thereafter, rats anesthetized with sodium pentobarbitol (50 mg/kg), were perfused with (1) saline, (2) 0.5% glutaraldehyde plus 2% paraformaldehyde in phosphate buffer, followed by (3) 10% sucrose. Sections (40 μm) were mounted on slides, processed with TMB, and examined with darkfield or fluorescence microscopy.

When FB was injected into thoracic spinal cord, retrogradely labeled neurons in the ventrolateral medulla were present in nucleus retroambiguus and RVL. Labeled neurons, in both cases, lay within a field of HRP-labeled terminals. In RVL, FB-labeled neurons occupied approximately the medial half of the terminal field from the NTS. Neurons were also retrogradely filled with HRP from the NTS; these were a separate population from those projecting to spinal cord.

Neurons labeled with FB after cervical vagus applications were found in the dorsal vagal nucleus (DMV) and nucleus ambiguus columns. Labeled neurons in the rostral DMV and ventrolateral medulla, including those innervating the heart , were surrounded by HRP-labeled fibers.

These data demonstrate that NTS projects directly to: (1) regions of RVL innervating thoracic intermediolateral column, and (2) vagal efferent neurons. These pathways may be the anatomical substrate of medullary visceral reflexes, including the baroreceptor reflex. The technique of combining anterograde transport of HRP with retrograde transport of FB in a single experiment is well suited for studying disynaptic pathways in the brain at the light microscopic level.

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209.11 Sleep state modulation of transient hypertension elicited by central amygdaloid nucleus stimulation. R. B. Trelease, R. M. Harper and R. C. Frysinger. Department of Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

Stimulation of the central nucleus of the amygdala (ACE) results in potent cardiovascular changes, and this nucleus projects to cardioactive areas in the parabrachial pons and to the nucleus tractus solitarius. Given that cardiac-related neurons in the pons alter their discharge pattern with sleep state, the ACE may contribute to state-related cardiovascular fluctuations.

Cats were instrumented under pentobarbital anesthesia with electrodes to record sleep state, EKG, and diaphragmatic EMG. Stimulation electrodes were placed in the ACE and a pressure monitoring catheter was advanced into the aorta from the femoral artery. After 1 week of recovery, cats were placed in a behavioral chamber for monitoring of blood pressure during waking and sleep states. Elevation of blood pressure was obtained by short (maximum effect with .3 to .5 sec duration) 90 Hz stimulus trains to the ACE. The cardiovascular responses were determined by assessing the maximum systolic and diastolic rise for 30 sec following stimulation, and the cardiac R-R interval responses over the same time period.

Both the maximum systolic and diastolic pressure rises were diminished during Quiet Sleep from Waking values. In all states, the initial 1-2 sec of hypertension was accompanied by a brief tachycardia, followed by a sustained bradycardia with accentuated respiratory arrhythmia (Fig. 1). There were apparent state-related differences in the magnitude of evoked respiratory arrhythmia between states. Similar cardiovascular effects were obtained with train frequencies as low as 5 Hz. Pulse rates below 5 Hz produced no rise in blood pressure, although other respiratory and behavioral effects were elicited.

Stimulation of the ACE exerts a powerful effect on cardiovascular dynamics. The present findings suggest that part of the differential cardiovascular responses observed during sleep states could result from modulation of ACE influences on the brainstem.

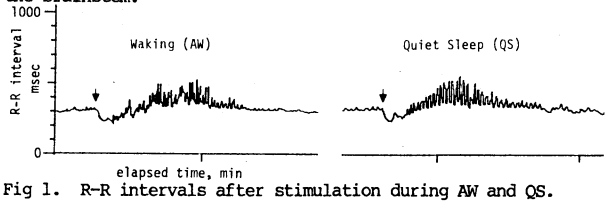

Fig 1. R-R intervals after stimulation during AW and QS.

209.12 MODULATION OF MAGNOCELLULAR ACTIVITY BY ANGIOTENSIN II. L. D. Mitchell, S. Bellin, K. Barron, M. Brody and A. K. Johnson. Department of Psychology and The Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

The supraoptic-hypophyseal tract is a primary system for the synthesis and release of vasopressin. Angiotensin II (AII) has been shown to release vasopressin when injected into the cerebral ventricles (IVT). However, intravenous (IV) AII injections have not produced consistent results. The present studies were conducted to examine the effects of AII delivered by either route on the unit activity of magnocellular neurons. Rats were prepared with intracranial cannulas to insure delivery of drugs to the left jugular vein, femoral vein, and femoral artery for systemic injections and arterial pressure recordings. A ventral approach permitted recordings from the SON without violating the ventricular-SON partition. Magnocellular neurons were electrophysiologically identified.

Intraventricularly injected AII (50 ng) increased the firing of 8 neurons (latency 4-40 sec), decreased firing in one, and in 4 units no change was observed. The specific antagonist saralasin (500 ng) alone reduced the firing of 5 units (latency 2-10 sec). In 2 cases saralasin blocked ongoing AII excitation. Lesioning an AII sensitive region, the anteroventral third ventricle (AV3V), blocked the action of AII on magnocellular neurons. In 4 cases, spontaneously active neurons failed to respond to AII. In one case, a continuous recording demonstrated that the response to central AII was markedly reduced after the AV3V was damaged.

In intact rats, AII IV (100 ng-10 μg) inhibited 12 units (latency 4-30 sec for 20 sec-3 min) while raising blood pressure (range 150-200 mmHg) one unit failed to respond. The systemically acting pressor agent phenylephrine (PhE) (10 μg-40 μg/kg) inhibited 7 neurons with a similar rise in blood pressure. In rats with high pressure baroreceptors removed (SAD) 23 neurons were recorded. AII (3 μg) and PhE (150 ng) raised blood pressure to approximately 140 mmHg. AII increased firing of 15 of 20 SON neurons. PhE administered to the debuffered rats produced little or no inhibition. Lowering the blood pressure with nitroglycerine was without effect on unit firing as well.

We conclude that circulating AII has a CNS excitatory action on SON magnocellular neurons, but this action is inhibited by the accompanying pressor response via baroreceptor input.

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210.1 THE DISTRIBUTION OF CROSS CORRELATED ACTIVITY BETWEEN GAMMA MOTONEURONES TO HIND LIMB MUSCLES IN THE CAT. P.H.Ellaway* and K.S.K.Murthy. Dept. of Physiol., Univ. Coll. London, London WC1E 6BT, England* and Division of Neurosurgery, Univ. of Texas Med. Sch., Houston, Texas 77030.

The naturally occurring discharges of γ motoneurones to gastrocnemius muscles in the hind limb of decerebrated, spinal cats invariably show a degree of correlation (Ellaway et al., J. Physiol. in press). Typically a γ motoneurone has a peak of increased probability of firing at the moment of discharge of another γ motoneurone which decreases to a control level within 5 to 10 msec. The ratio of the peak increased probability of firing to the control level in the cross correlation has ranged from 1.2 to 8.3 with a modal value between 1.5 and 2.0.

It has been proposed (Moore et al., Biophys. J.10, 1970; Knox & Poppele, J. Neurophysiol. 40, 1977; Kirkwood, J. Neurosci. Meths 1, 1979) that this form of short term synchronisation is a consequence of shared synaptic inputs to a population of neurones. We are now examining the degree of correlation between γ motoneurones to different muscles in an attempt to further our understanding of the mechanisms controlling their discharge.

Cats were decerebrated intercollicularly under halothane anesthesia and spinal cord section made at either C1 or T9. Anesthesia was discontinued, the animals were paralysed with Flaxedil and artificially respired. Discharges of γ motoneurones, identified by axonal conduction velocity (15-40 m/sec), were recorded from dissected filaments of peripheral nerve.

The degree of correlated activity between pairs of neurones to gastrocnemius medialis (gm) and lateralis (gl), and soleus (sol) was assessed. Correlations between neurones to these different parts of triceps surae were usually similar in form and as intense as those within one muscle head. However, in one cat the cross correlations between gm and gl consisted of dips compared with the more usual peaks present within a muscle head. In another cat correlations were present between gm pairs and between sol pairs but not for gm/sol pairs of γ motoneurones unless intentional stimulation of the skin of the heel was provided. No correlations were observed for homologous muscles across the cord.

Correlations within flexor hallucis longus (fhl), a synergist of triceps surae, were present for only 7 out of 16 pairs of γ motoneurones. Neither the discharges of neurones contributing to the 7 pairs, or of the others, were correlated with the discharges of any gm units.

Our interpretation of these results is that some of the synaptic inputs producing discharges of γ motoneurones in closely synergistic muscles must have different origins at the spinal segmental level.

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210.2 FREQUENCY AND DISTRIBUTION OF AXON COLLATERALS FROM UPPER CERVICAL SPINAL MOTONEURONS. S. A. Keirstead*, P. K. Rose, and S. J. Vanner*. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Electrophysiological studies have shown that the presence of recurrent inhibition in upper cervical spinal motoneurons is related to the target muscle of the motoneuron (Rapoport, S., J. Physiol. 289: 311, 1979). For example, volleys arriving over the ventral roots rarely elicited IPSP's in motoneurons innervating the dorsal neck muscles, splenius (SP), biventer cervicis (BC), and complexus (CM). In contrast, larger recurrent IPSP's were evident in the majority of motoneurons innervating the shoulder muscle trapezius (TR). The purpose of this study was to determine if these physiological differences can be attributed to corresponding variations in axon collateral frequency and distribution.

Motoneurons were antidromically identified and intracellularly stained with horseradish peroxidase. Most motoneurons, regardless of their target muscle, had at least one axon collateral. Reconstructions of these collaterals from serial histological sections showed that the axon collaterals of each motoneuron species were primarily distributed within and around the parent motoneuron nucleus. All of the collaterals were distinguished by numerous swellings (which may be analogous to presynaptic terminals) which were particularly abundant within the motoneuron nuclei. Quantitative analysis revealed that each axon collateral system usually gave rise to 40 to 90 swellings. There were no major differences between the collateral systems of motoneurons innervating dorsal neck muscles and the trapezius muscle.

These results indicate that the weak recurrent inhibitory effects observed in dorsal neck muscle motoneurons cannot be attributed to poorly developed axon collateral systems. Moreover, the weak recurrent inhibitory connections are not a simple consequence of the unusual distribution pattern of the collaterals since axon collaterals from trapezius, which are subject to recurrent effects, were also located in and around the parent motoneuron nucleus.

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210.3 SPECIFIC AND POTASSIUM MECHANISMS INVOLVED IN THE PRIMARY AFFERENT DEPOLARIZATION OF GROUP Ia FIBERS IN THE SPINAL CORD OF THE CAT. P. Rudomin, L. Vyklický*, I. Jiménez* and M. Solodkin*. Centro de Investigación del I.P.N. México 14, D.F.

Primary afferent depolarization (PAD) has been attributed to the activation of GABAergic axo-axonic synapses with afferent fiber terminals, and to accumulation of potassium ions in the extracellular space (K^+) resulting from neuronal activity. Although both mechanisms appear to coexist, the extent to which they participate in the generation of the PAD produced by the activation of a given pathway has not been elucidated.

The aim of this study was to correlate the negative potential DC shifts and changes in extracellular K^+ produced in the spinal cord by stimulation of specified types of cutaneous and muscle afferents with the changes in the PAD of single Ia fiber terminals. Experiments were made in cats anesthetized with pentobarbital (35 mg/kg), paralyzed and artificially respired. K^+ transients were measured with double barrelled K^+ specific microelectrodes. PAD of single fibers was inferred from the changes in their activation threshold estimated from the current necessary to keep a constant probability of antidromic responses.

High frequency stimulation (50-200 Hz for 10 to 20 sec) of the posterior tibial nerve (PT) at strengths ranging from 2 up to 100 times threshold produced negative DC potential shifts and K^+ increases which were maximal (up to 7 mmol/l) in the dorsal horn and decayed gradually in deeper regions. This contrasted with the very small increases in K^+ concentration (less than 0.3 mmol/l) produced in the intermediate nucleus by similar repetitive stimulation of group I afferents in the posterior biceps and semitendinosus nerve (PBSt), which reduced very effectively the activation threshold of nearby single Ia gastrocnemius (GS) fibers. In addition, high frequency stimulation of the sural nerve, which produced larger increases in K^+ concentration, had no effect on the activation threshold of the Ia GS fibers, although it inhibited the PAD produced by PBSt conditioning volleys. On the other hand, the excitability increases produced by stimulation of the PT nerve correlated with the DC potential shifts and the increases of K^+ recorded at the site of excitability testing.

It is suggested that the PAD of group Ia fibers produced by stimulation of group I muscle afferents is due to the activation of specific neuronal pathways which do not lead to a significant accumulation of K^+. However, the PAD of group Ia fibers produced by stimulation of the PT nerve is likely to be due to the contribution of both a specific and a K^+ dominant component.

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210.4 THE NEURONAL NETWORK WHICH MEDIATES COLLATERAL INHIBITION OF THE GOLDFISH MAUTHNER CELL, John T. Hackett and Donald S. Faber, Dept. of Physiol., Univ. of Virginia, Charlottesville, VA 22908 and Div. of Neurobiol., SUNYAB, Buffalo, NY 14214.

A single impulse evoked in a goldfish Mauthner (M) cell by acoustic or visual stimuli results in a rapid unilateral tailflip or startle reflex. The discharge of either M-cell is followed by a powerful feedback inhibition mediated by a recurrent collateral network (Furakawa & Furshpan, '63). The collateral inhibition consists of (1) an initial short latency electrical component which is characterized by an extracellular positivity recorded in the M-axon cap and has been named the extrinsic hyperpolarizing potential (EHP) and (2) a subsequent, inhibitory postsynaptic potential (IPSP) recorded from the M-cell soma. Both inhibitions are generated by impulses in identifiable medullary neurons (Korn & Faber, '76). Since our studies have shown that several cranial premotor neurons (CPNs), connected in parallel, are interposed between the M-axon and motoneurons that innervate the eye, jaw and opercular muscles (Hackett & Faber, '81), we have explored the possibility that these CPNs also evoke the feedback inhibition of the M-cells.

Experiments were performed on goldfish 10-15 cm in length that were anesthetized and perfused through the mouth with aerated water. Two microelectrodes were used to obtain simultaneous recordings from a CPN and from either the M-cell soma, its axon cap, or its inhibitory neurons. Impulses evoked directly in CPNs were followed by EHPs in the axon cap (latency 1.3 ± 0.3 ms, n=6) and, in separate experiments, by chloride-dependent IPSPs in the M-cell soma (latency 1.8 ± 0.5 ms, n=9). Simultaneous intracellular recordings have also shown that the inhibitory neurons are postsynaptic to some CPNs. Intracellular stimulation of the latter produce short latency depolarizations of the inhibitory neurons. Synaptic transmission between the two units appears to be chemically mediated, because it fatigues at low stimululation frequencies and is unidirectional.

The collateral network is organized such that an impulse in one M-axon leads to inhibiton of both M-cells, while it is now established that the terminal fields of an inhibitory neuron is unilateral (Korn, Triller & Faber '78). Thus, it was previously hypothesized that either the M-axon collaterals crossed the midline or the inhibitory neurons were activated by excitatory ones receiving bilateral inputs. Our evidence supports the notion that both M-axons converge on individual CPNs which in turn excite the inhibitory neurons. This network may serve as model for the integration of recurrent inhibition in other motor systems.

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210.5 EFFECT OF ANTIDROMIC ACTIVATION OF BULBOSPINAL RESPIRATORY NEURONS ON PHRENIC NEURAL DISCHARGE. D.R. McCrimmon*, D.F. Speck* and J.L. Feldman. Departments of Physiology and Anesthesia, Northwestern University Medical School, Chicago, IL 60611.

These experiments examined the effects on respiratory neural outflow elicited by synchronous activation of bulbospinal respiratory pathways. Experiments were conducted in chloralose-urethane anesthetized, gallamine paralyzed and artificially ventilated cats. Descending respiratory axons were activated at the C2 level using either monopolar or bipolar stimulation (25-100 uA, 50-100 us). Activation of descending inspiratory pathways was confirmed by recording antidromic invasion of single, inspiratory modulated units in either the nucleus tractus solitarius or the nucleus retroambigualis. Both ipsi- and contra-lateral phrenic nerve activities were recorded. Single stimuli delivered to the spinal cord elicited a short latency (2-4 msec) orthodromic excitation of the ipsilateral phrenic nerve during inspiration. This excitation lasted approximately 2 msec. A smaller amplitude short latency (3-4 msec) activation of the contralateral phrenic nerve also was elicited during the inspiratory phase. In both the ipsi- and contra-lateral phrenic nerves, the latency decreased as inspiration progressed. Continuous trains of stimuli (50-100 uA, 100 us, 4-100 Hz) or phrenic gated trains delivered during every fourth inspiratory cycle did not alter the duration of either inspiration or expiration. Since synchronous activation of a portion of a rhythm generator would be expected to phase shift or reset the rhythm, we conclude that the bulbospinal respiratory neurons are not responsible for respiratory rhythm generation. Also it is unlikely that these bulbospinal neurons have collateral axons which affect a respiratory phase switching mechanism. (Supported by NIH grants NS-17489, HL-00554 and HL-06331).

210.6 EVIDENCE FOR MOTONEURON COLLATERAL INPUT TO VSCT NEURONS. J. H. Kim,* G. W. King, T. J. Ebner, J. R. Bloedel. (SPON: R. Gumnit). Departments of Neurosurgery & Physiology, University of Minnesota, Minneapolis, 55455.

The important role played by the cerebellum in regulating motor behavior is consistent with the numerous inputs to this structure from the collaterals of neurons descending from the cerebral cortex. However, the existence and importance of inputs from the final component of the motor system, the motoneuron, has not been adequately investigated. The purpose of this study was to demonstrate that ventral spinocerebellar tract (VSCT) neurons receive inputs from collaterals of motoneurons and to characterize the responses evoked by this system. In cats anesthetized with alpha chloralose, the L7 ventral root was cut and mounted on a bipolar stimulating electrode. VSCT neurons in the same segment were identified by their antidromic activation from the contralateral superior peduncle. Post-stimulus time histograms (PSTHs) were constructed from the extracellularly recorded responses of VSCT neurons to ipsilateral ventral root (VR) stimulation. The stimulus intensities required to evoke the threshold response were compared with the threshold for the field evoked by the antidromic activation of alpha motoneurons. Of 95 VSCT cells studied (25 cats), 78 neurons were excited by an ipsilateral VR volley. In 37 cells tested, the threshold for the activation of VSCT cells varied greatly, ranging from 1.2 to 20 x threshold for the antidromic field. However, the majority of neurons (26/37) were excited by VR volley at intensities less than 5 x the antidromic threshold. The response to ventral root stimulation usually consisted of a burst of action potentials with a latency of approximately 2 msec. Occasionally this burst of activity was followed by a reduction in impulse activity and/or a second burst with latencies of 20.2 ± 7 msec. Although the short latency and low threshold responses of most VSCT cells to ventral root stimulation strongly suggest that they are not evoked by the small diameter afferents in the ventral roots, an additional experiment was done to more directly rule out this possibility. Ventral root afferents were interrupted by carefully dissecting and cutting the dorsal root just distal to the dorsal root ganglion, interrupting ventral root afferent fibers. VSCT neurons in this segment were readily activated by stimulating the nerve distal to this cut. The characteristics of these responses were very similar to the responses when the dorsal root was intact. This study demonstrates that VSCT neurons receive a strong excitatory input from the collaterals of motoneurons, providing the cerebellum with information of the activity in the final stage of the motor system. Supported by NIH Grant #2R01-NS 09447-10.

210.7 MOTOR TRACT EVOKED POTENTIALS

W. J. Levy and D. H. York, Division of Neurosurgery, University of Missouri-Columbia, Columbia, Missouri 65212

To provide a better monitoring system of motor function in the spinal cord we are developing motor tract evoked potentials. This method uses direct stimulation of the spinal cord between the intermediolateral sulcus and the dentate ligament, the area overlying the motor tracts. A one milliamp, one millisecond bipolar square wave signal is delivered to this area from 2 mm platinum ball electrodes. Recording was done from the dura or epidural space at several levels of the spinal canal. A 100 meter per second signal was obtained which did not disappear with lesioning of the dorsal columns, anterior half of the spinal cord, or area of the dorsal spinocerebellar tract. It did disappear with lesioning of the motor area. The spinal cords from these animals were sectioned at 50 µ after Hematoxylin and Eosin staining. The signal is a negative-positive-negative wave. This test has been used in both cats and man. It can produce motor responses in the distal extremities in man and cat at the appropriate stimulation frequency.

In the ongoing human study, 7 patients were studied who were undergoing a cervical laminectomy because of either a tumor or cordotomy for intractable pain. All patients were male with a mean age of 62 years. Stimulating electrodes consisting of platinum ball electrodes were placed on the dorsal columns and more laterally overlying the corticospinal tracts. Recordings were made from a deep needle electrode inserted at T9-12 vertebrae referenced to a surface subcutaneous needle electrode. Potentials were amplified by 10^4 over a bandpass of 1-3000 Hz and averaged with a Nickolet CA1000 signal averager for 128-256 stimulus presentations. Each stimulus was square wave constant current pulse 0.5-2.0 mA, 0.1-0.2 msec, 1-3 sec. The potential recorded after motor tract stimulation was consistently greater than 90 m/sec (range 93-124 m/sec), in contrast to the dorsal column antidromically evoked potential which varied from 64-75 m/sec. In one patient who had tumors affecting the dorsal columns, the conduction velocity recorded across the zone of pathology decreased to 27 m/sec although the motor potential remained at 93 m/sec.

We have observed loss of the motor signal in a patient with motor deficits when the somatosensory evoked potential was unimpaired. These results suggest that direct assessment of motor tract conduction velocity is possible during surgery and may also be of value in the prevention of and prognosis of spinal cord injury.

210.8 AFFERENT PROJECTIONS OF THE PARASYMPATHETIC NUCLEI IN THE RAT. J. E. Nicholson and C. M. Severin. Department of Anatomy, Univ. of Texas Medical Branch, Galveston, TX. 77550.

The afferent projections of the four brain stem parasympathetic nuclei were examined in the rat using the horseradish peroxidase (HRP) and autoradiographic techniques.

Injections of a 33-50% solution of HRP were made into each of the parasympathetic nuclei in separate animals. The rats were sacrificed following a survival period of 24-48 hours and the brains processed with either diaminobenzidine tetrahydrochloride or tetramethylbenzidine.

Potential sources of afferent fibers so identified were subsequently injected with a mixture of tritiated leucine and proline and the tissue processed for autoradiography.

Confirmed sources of input to the dorsal motor nucleus of the vagus nerve included the ipsilateral nucleus of the solitary tract and the paraventricular nucleus of the hypothalamus (PVH). Afferent projections to the inferior salivatory nucleus were verified from the medial and superior vestibular nuclei and from the PVH. A bilateral connection was seen from the nucleus locus coeruleus to the inferior salivatory nucleus.

Areas which projected to the superior salivatory nucleus included the medial and superior vestibular nuclei, the locus coeruleus, and the PVH. Although projections were bilateral, the ipsilateral pathways were more substantial. Finally, projections were confirmed to the Edinger-Westphal nucleus from the parabrachial nucleus, locus coeruleus, nucleus of the posterior commissure, lateral habenular nucleus, and from the lateral and paraventricular hypothalamic regions. No attempt was made to differentiate ipsilateral from bilateral projections in the case of the Edinger-Westphal nucleus, as HRP injections into the midbrain generally involved both nuclei and resulted in bilateral labelling.

210.9 THE EFFECTS OF INTRATHECAL ADMINISTRATION OF CLONIDINE ON NEURONAL ACTIVITY IN NUCLUES LOCUS COERULEUS. J. Marwaha, R.L. Commmissaris*, J.H. Kehne*, and M. Davis. Dept. Pharmacol., Univ. Indiana Sch. Med., Terre Haute, IN 47809, and Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508.

The spinal cord is becoming increasingly identified as a primary site for drug action. This concept has resulted partly from the development of the intrathecal (IT) technique, which permits drug infusion directly into the spinal subarachnoid space. Effects observed after IT drug administration are usually interpreted to be mediated by primary sites/mechanisms residing in the spinal cord.

To evaluate whether supraspinal areas might be affected by the intrathecal administration of lipophilic drugs, the spontaneous discharge rate of single noradrenergic neurons in the nucleus locus coeruleus (LC) in the brainstem was investigated following intrathecally administered clonidine.

Previous studies (Svensson et al., 1975) have shown that low doses of systemically (10 ug/kg, i.v.) or iontophoretically administered clonidine consistently, rapidly and reversibly inhibit the spontaneous firing of noradrenergic neurons in the LC of chloral hydrate anesthetized rats. In the present study, low doses of intrathecally administered clonidine (3-10 ug; approximately 6-25 ug/kg) also consistently, rapidly and reversibly inhibited LC neuron firing. This potent inhibitory effect of IT clonidine was still observed in spinally transected rats, ruling out a neuronal feedback mechanism from the spinal cord to the LC. Although IT administration of the lipophobic α-adrenergic antagonist phentolamine did not alter the inhibitory effects of IT clonidine, intraventricular administration of phentolamine blocked the effects of IT clonidine on LC activity. Oxymetazoline, a lipophobic α_2-adrenoceptor agonist, had no effect on LC neuron firing after IT administration. However, oxymetazoline potently inhibited LC neuron firing after either intraventricular or iontophoretic application.

Our results suggest that the inhibitory effects of IT clonidine on LC neuron firing result from the rapid movement of the drug out of the spinal cord to supraspinal sites. These data indicate that caution should be exercised before exclusively identifying the spinal cord as the site of action following IT administration of lipophilic drugs.

Reference: Svensson, T.H., Bunney, B.S., and Aghajanian, G.K. (1975) Brain Res., 92: 291-308.

210.10 MEMBRANE POTENTIAL DEPOLARIZATION OF mPRF NEURONS DURING BEHAVIORAL STATE CHANGES FROM SYNCHRONIZED TO DESYNCHRONIZED SLEEP IN NATURALLY SLEEPING CATS. R.W. McCarley and K. Ito. Harvard Medical School, Boston, MA 02115

We have hypothesized that the bringing to firing threshold of medial pontine reticular formation (mPRF) neurons occurs as a critical event in generation of some desynchronized (D) sleep phenomena, such as rapid eye movements and PGO waves. To test this hypothesis, we have performed chronic intracellular recordings in unanesthetized, undrugged cats during naturally occurring sleep-wake cycles. Cats were prepared with electrodes for recording state variables and for microstimulation (0.2 mS, < 80 μA) in contralateral mPRF and ipsilateral mesencephalic and bulbar (BRF) reticular formation. All recording and stimulation sites were histologically verified by marker lesions and in some instances by intracellular injection of HRP. We recorded 28 mPRF neurons with membrane polarization (mp) levels more negative than 45 mV in synchronized sleep (S) and monitored in contiguous segments of S, transition period (T; PGO waves without other D signs), and D.

As the animal passed through S and approached the onset of T, there was usually a graded, more or less continuous depolarization. Mean depolarization from the low point in S to the initial portion of T was 4.0 mV (± 2.5 mV, range +2.5-11 mV). During T, membrane depolarization continued with a mean increase from T to D of 3.7 mV (±1.9 mV, range +0-7 mV). Spontaneous discharge frequently began during T and increased pari passu with increasing depolarization. D was characterized by virtual storms of increased spontaneous, depolarizing PSP activity, which tended to occur in phasic runs with increased spontaneous discharge activity. Usually following spikes was an after-spike hyperpolarization or hyperpolarizing PSPs; these appeared to limit maximal discharge frequency. There was a state-dependent S to D increase in excitability of monosynaptic responses to mPRF microstimulation, including a four-fold increase in probability of monosynaptic latency spike generation.

These data provide the first evidence that depolarization of mPRF neurons precedes the earliest electrographic signs of D and increases through T to D. This contrasts with a later appearance of depolarization reported in BRF neurons and suggests that mPRF neurons are either closer to the generation site of D sleep or themselves may play an active role in this. The monosynaptic stimulation data indicate that mPRF neurons increase in excitability on transition from S to D. All of these data suggest that a consistent event in D sleep generation is increasing the excitability of and the bringing to firing level of mPRF neurons.

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210.11 TIME COURSE OF GROUP Ia SYNAPTIC CURRENT TRANSIENTS IN ALPHA MOTONEURONS. G. W. Sypert, J. B. Munson and W. Rall. Depts. of Neuroscience and Neurological Surgery, Univ. Florida Coll. of Med., Gainesville, FL 32610 and National Institutes of Health, Bethesda, MD 20205.

Computational experiments were reviewed using the Rall mathematical neuron model proposed (1) to predict the temporal parameters of theoretical EPSPs generated by smooth synaptic current transients localized in one of ten neuronal somadendritic compartments. The results provide quantitative predictions of the EPSP shape indices which can be used for accurate comparisons with experimentally observed EPSPs. The shape indices (10-90% rise time and half-width) of theoretical EPSPs were compared with experimentally observed spike-triggered averaged group Ia-motoneuron single fiber EPSPs selected for simple terminal potentials (see page 332 of ref. 3), which are assumed to correspond to highly localized terminations on the motoneuron surface (2,3). The shape indices of these experimentally observed EPSPs were found to agree best with a theoretical EPSP shape index locus computed with a briefer input time course than the "fast" input of (1); this brief input peaks at 0.01τ and has a half-width of 0.025τ, where τ is the passive membrane time constant of the neuron; it corresponds to α = 100 in the notation of Jack and Redman (4). Because this experimental EPSP sample had an average τ close to 5 msec, the average Ia synaptic current of these synapses is inferred to peak in 50 μsec, with a half-width of 125 μsec. It is noteworthy that this agreement between theoretical and experimental shape indices extends over soma-dendritic input locations from compartment 1 to 8 of the ten compartment model (corresponding to electrotonic distances from 0 to 1.4) for the same very brief input time course. This implies independence of synaptic current time-course from soma-dendritic location of the synapse, for this selected EPSP sample, which explicitly excluded multiple synapses with latency spread. This reinforces earlier findings (4,5) that such EPSP shape variety can be explained by the passive electrotonic spread from different soma-dendritic input locations, for a single brief synaptic current time course. With regard to shape index comparisons, we note that the 10-90% rise time equals very nearly 55% of the time from foot to peak of the theoretical EPSPs of this series (α = 100).

References: (1) Rall, J. Neurophysiol., 30:1138, 1967; (2) Munson and Sypert, J. Physiol. 296:315, 1979; (3) Ibid. :329, 1979; (4) Jack, Miller, Porter and Redman, J. Physiol., 215:353, 1971; (5) Rall, Burke, Smith, Nelson and Frank, J. Neurophysiol., 30:1169, 1967.

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210.12 THE REPETITIVE STIMULATION OF CUTANEOUS AFFERENTS PRODUCES AN ACCUMULATIVE AND PROGRESSIVE INCREASE OF THE POST-TETANIC POTENTIATION (PTP) IN POLYSYNAPTIC REFLEXES IN SPINALIZED CATS. A. Fernández-Guardiola, J. M. Calvo*, F. Pellicer* and R. Alvarado* Unidad de Investigaciones Cerebrales, Instituto Nacional de Neurología y Neurocirugía. Insurgentes Sur # 3877, 14410 México, D. F.; Facultad de Psicología, U.N.A.M.

Post tetanic potentiation, when provoked in structures such as the hippocampus, causes a long term synaptic facilitation, which has been associated to learning and memory phenomena and to the kindling effect (Goddard, 1967). In a previous study, we found an accumulative increase of mono and polysynaptic PTP in spinalized cats (Fernández-Guardiola et al., 1981). In the present one, we continue the research on the mentioned effect in 33 spinalized cats, stimulating the sural nerve at 100 Hz during 3 sec (pulses of 0.3 msec) every 20 min, with currents ranging from 200 to 600 μA. After a 3 hour control, the first 8 post-tetanic responses (0.2 sec single tests) recorded at the ventral roots (L6, 7, S1) were averaged. The field potentials, were recorded with a mental microelectrode (1-5μ) at different depths (400-1000u) in the gray matter of the spinal chord; the afferent volleys in the dorsal surface of the spinal chord were also recorded and averaged.

The periodical activation of the sural nerve provoked an amplitude increase, a shortening and time lock in the polysynaptic responses latencies, which reached their maximal amplitude at 3-4 hours. The sural successive tentanizations came together with an increase of the spontaneous and post-stimulus activity of the interneurones of Rexed's layers 2,3 and 4, as well as the ventral root discharge, at the same time the field potentials increased their amplitude, and an early component appeared, which grew and shortened its latency more than 25%, as related to the first PTP. This early field potential, together with the late polysynaptic ones were increased more than 300% whith naloxone intra-venously injected (0.4 to 0.8 mg/Kg), and the latency which had been shortened 10% during the first PTP decreased and additional 17% with the drug.

We can conclude that the spinal chord presents the long term PTP facilitation when adequately stimulated, using a method similar to that of amigdaline or hippocampal kindling. This potentiation mechanism could be attributed to the progressive diminution of an inhibitory effect of enkephalinergic interneurons. (naloxone effects).

211.1 RECONSTITUTION OF A FUNCTIONAL MAMMALIAN SODIUM CHANNEL FROM PARTIALLY PURIFIED COMPONENTS. J. A. Talvenheimo*, M. M. Tamkun* and W. A. Catterall. Dept. of Pharmacology, Univ. of Wash., Seattle, WA 98195.

Voltage-sensitive sodium channels were solubilized from rat brain using Triton X-100. The saxitoxin (STX) binding component of the channel was purified 60- to 80-fold by ion exchange chromatography, followed by wheat germ agglutinin chromatography, as described by Hartshorne and Catterall (Proc. Natl. Acad. Sci. U.S.A. 78:4620-4624). The purified STX receptor was incorporated into egg phosphatidylcholine (PC) vesicles by adding PC in Triton X-100, then removing the detergent with Bio-Beads SM2. Approximately 40-60% of the solubilized STX binding sites were recovered in PC vesicles. Following reconstitution, the STX receptor regained thermal stability and exhibited STX binding properties identical to those of synaptosomal membranes. Solubilization of the vesicles showed that about 70% of the reconstituted STX binding sites are located on the outer vesicle surface, while 30% of the sites, revealed after detergent treatment, are located on the inner surface. ^{22}Na influx into vesicles containing the partially purified STX receptor was measured with a 10-fold sodium gradient (in > out) imposed across the vesicle membrane. Under these conditions, 100 μM veratridine (VER) stimulated ^{22}Na influx 3- to 4-fold. Seventy per cent of the VER-stimulated ^{22}Na influx, measured at 20 sec, was blocked by external tetrodotoxin (TTX). This result is consistent with our data showing that 70% of the STX binding sites are oriented outward in these vesicles. VER-enhanced ^{22}Na influx was completely blocked when TTX was present both internally and externally, or when the local anesthetic tetracaine was added, indicating that all of the VER-stimulated ^{22}Na flux occurs via active sodium channels. The $K_{0.5}$ of 11 μM for VER enhancement of ^{22}Na flux through the reconstituted channel agrees closely with the value of 13 μM measured for VER activation of synaptosomal sodium channels (Tamkun and Catterall, Mol. Pharmacol. 19:78-86). Moreover, TTX inhibited the VER-stimulated ^{22}Na influx through reconstituted channels with a K_i of 10 nM, in excellent agreement with the K_i for TTX inhibition of ^{22}Na flux in intact synaptosomes. VER did not stimulate ^{22}Na influx in PC vesicles formed with STX receptor denatured by incubation at 36°C. These results strongly suggest that the partially purified STX receptor contains, in addition to the STX and VER binding sites, the channel components required for sodium flux.

211.2 SELECTIVE PHOSPHORYLATION OF THE α SUBUNIT OF THE SODIUM CHANNEL BY cAMP DEPENDENT PROTEIN KINASE. M.R.C. Costa*, J.E. Casnellie* and W.A. Catterall. University of Washington, Seattle, WA 93195.

Partially purified preparations of the rat brain sodium channel obtained by column chromatography on DEAE-Sephadex and WGA-Sepharose were phosphorylated with [$\gamma^{32}P$]ATP in the presence of the purified catalytic subunit of cAMP-dependent protein kinase. In these preparations the α subunit of the sodium channel can be clearly distinguished in the protein staining pattern after NaDodSO$_4$ gel electrophoresis as a broad protein band of $M_r \sim 270{,}000$. Autoradiographic analysis showed that ^{32}P was preferentially incorporated into the α subunit band. Sodium channels from this preparation were further purified by sedimentation through a sucrose gradient and fractions taken along the gradient were phosphorylated in the presence of catalytic subunit of protein kinase. After this additional purification step the α and β subunits of the sodium channel constitute 65% of the total protein in the peak fractions. The migration of the saxitoxin binding activity across the gradient coincided with the migration of the heavily phosphorylated α subunit band of $M_r \sim 270{,}000$. In these experiments 3-4 moles of ^{32}P were incorporated per mole of saxitoxin receptor.

When partially purified sodium channel preparations were incubated with [$\gamma^{32}P$]ATP alone, there was also a preferential incorporation of ^{32}P into the α subunit. Among 3 preparations tested, the amount incorporated after 1 min incubation was 0.04-0.07 moles ^{32}P per mole of α subunit. This endogenous phosphorylation was stimulated 2-fold by cAMP and inhibited 50% by the specific inhibitor of the catalytic subunit of cAMP-dependent protein kinase. These results indicate that a cAMP-dependent protein kinase copurifies with the sodium channel and phosphorylates the α subunit selectively.

The initial rate of phosphorylation of α by the catalytic subunit of protein kinase is comparable to that of the synthetic peptide analog of the phosphorylation site of pyruvate kinase, one of the best substrates for cAMP-dependent protein kinase. Taking the values reported for the saxitoxin receptor density (4 pmol/mg protein) and cAMP-dependent protein kinase concentration (30 pmol/mg protein) in synaptosomes and extrapolating linearly from our initial rate of phosphorylation of α (V_{max}/K_m = 2.9 $l \cdot min^{-1} \cdot mg^{-1}$), we estimate that all sodium channels in the synaptosomes could be phosphorylated in 1 sec, if only 10% of the cAMP-dependent protein kinase were activated. Phosphorylation in this time range can play an important role in neuronal adaptive responses.

211.3 VISUALIZATION OF THE SODIUM CHANNEL AND ITS IMMUNOCYTOCHEMICAL LOCALIZATION. M.H. Ellisman, S.R. Levinson, W.S. Agnew, J. Miller, T.J. Deerinck*. Dept. of Neurosciences, Univ. of California, San Diego, Sch. of Med., La Jolla, CA 92093.

The tetrodotoxin binding protein (TTXR), a major component of the sodium channel, has been purified from the electric organ of the South American eel Electrophorus electricus. Highly purified preparations were examined by negative staining. Structures observed in preparations exhibiting the highest TTX binding tended to aggregate into ordered clusters with a unique ribbon-like conformation. The individual particles of these aggregates are elongated or rod-shaped, approximately 40A wide x 170A in length. Stereoscopic imaging of the 3-dimensional aspects of the structures revealed that the rod-like image is not an edge view of a flattened disc but represents a cylindrical structure. Individual rods in non-clustered forms were also observed but with greater frequency in preparations with lower specific activity. The dimensions of the particles suggest that they represent a protein core formed by perhaps one copy of the large glycopeptide previously identified as being part of the sodium channel. Antibodies to this protein were raised in rabbits and the specificity demonstrated by a highly sensitive radioimmunoassay and immunoprecipitation procedures. These antibodies were used to examine the distribution of the TTXR in the eel electroplax membranes and along myelinated nerve axons. The distribution of the TTXR was determined using the peroxidase-antiperoxidase technique at both the light and electron microscopic levels. In the electrocytes of the electric organ, only the innervated face showed staining in experimental material. The regions of electroplax plasmalemma which stained included the caveolae of the innervated surface while caveolae of the non-innervated surface did not stain. Thus, the innervated surface including caveolae exclusively contains the sodium channels. Along myelinated axons, staining was limited to the nodal zone of the node of Ranvier. The paranodal and internodal zones did not stain for the TTXR. Limited diffusion of primary IgG and subsequent reactants into paranodal and internodal sites was eliminated as a possible source of focal staining at nodes since mechanically demyelinated preparations also exhibited focal nodal staining. Thus, this TTXR component of the sodium channel appears to be located solely within the nodal zone of the node of Ranvier.

This work was supported by grants from NIH PHS14718, the Muscular Dystrophy Association and the National Multiple Sclerosis Society.

211.4 STOCHASTIC ANALYSIS OF THE KINETICS OF SINGLE SODIUM CHANNELS. R.W. Aldrich, D.P. Corey and C.F. Stevens. Dept. of Physiol., Yale Univ. School of Medicine, New Haven, CT 06510.

Single sodium channels can be readily studied in mouse neuroblastoma cells (clone N1E-115). The conductance is approximately 15 picosiemens and is similar to values obtained from other cell types. Stochastic analysis of opening events reveals details of the kinetics of the channels that cannot be seen using traditional voltage-clamp studies of sodium currents. A voltage-clamp step is applied repeatedly and single channel opening events are collected into distributions of: a) the probability of a channel being open versus time after the clamp step; b) the time after the clamp step until the first opening (first latency); and c) the duration of an opening. Further analysis allows the calculation of distributions that are conditional on another type of event, such as the distribution of durations of channels with a first latency greater than a specified time. These data can be compared with the predictions of various kinetic schemes for the channel.

Using this type of analysis, we have obtained the following results:

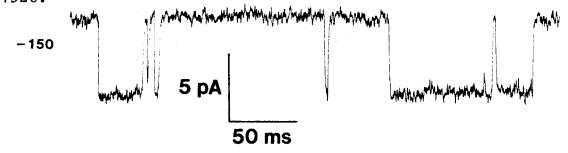
1. The distribution of durations cannot be fitted by a single exponential. This is not an artifact of unresolved closings, and it implies the existence of multiple populations of channels with the same conductance, or multiple open states of a single population of channels.
2. The first-latency distribution indicates that there is a significant probability that channels open for the first time well after the time of the peak probability of being open (which corresponds to the peak inward sodium current). This implies that activation is not near completion at the time of peak. Therefore, inactivation time constants obtained by fitting the declining phase of the current are overestimated due to continuing activation during this time.
3. The probability of a channel opening after it has closed is much less than the probability of initially opening following a voltage transition. This indicates that an open channel has a higher probability (and therefore a greater rate constant) for closing by passing to a state other than the one it opened from.

These properties could not have been detected by traditional voltage-clamp techniques. If results obtained from neuroblastoma sodium channels can be generalized to sodium channels in other cells, kinetic theories based on voltage-clamp studies of macroscopic sodium currents may have to be reevaluated in the light of single channel studies.

211.5 CONDUCTION AND KINETICS OF SINGLE ACETYLCHOLINE CHANNELS IN A CULTURED MAMMALIAN CELL LINE. G.A. Redmann, P.R. Adams and R.B. Clark. Dept. Physiology and Biophysics, Univ. Texas Medical Branch, Galveston, and School of Physiology and Pharmacology, Univ. New South Wales, Australia.

Acetylcholine channel currents from the cultured BC3-H1 cell line were examined in intact cells and excised membrane patches with the gigohm seal patch clamp technique. Currents were recorded via a pipette containing 100-400 nM acetylcholine and saline, which consisted of 150 mM of either NaCl or LiCl, 5.6 nM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10 mM glucose and 10 mM Na-HEPES, at pH 7.3. Bath solutions were similar, less the agonist. The I-V relation in 150 mM NaCl was approximately linear in intact patches, with a single channel conductance of 34.6±2.2 (SD) pS over the voltage range 0 to -150 mV. Currents reversed at an applied potential of +27.7±3.7 mV. Outward currents in the intact patch showed a slight rectification over the range +100 to +150 mV corresponding to a slope conductance of 26.9±4.9 pS. The I-V relation in 150 mV LiCl in intact patches was non-linear, being approximately 18 pS for inward currents over the applied potential range of -100 to -150 mV, and 30 pS for outward currents over the range +50 to +100 mV. The I-V for LiCl was linear in excised patches in symmetrical solutions, however, being 17±2 pS over the voltage range examined.

Single channel lifetimes in intact patches in 150 mM NaCl were exponentially distributed at any given applied potential. The mean lifetime was 24.0±25.5 mS at -150 mV, and 14.3±13.2 mS at -50 mV. Mean lifetime values were exponentially dependent on voltage, and showed an e-folding increase for 138 mV hyperpolarization, over the voltage range +100 to -200 mV. Single channel opening frequencies varied with agonist concentration, the number of channels in each patch, and membrane potential. At constant, low agonist concentration, normalized opening frequencies, from a range of 4-16/sec, showed an e-folding increase for approximately 220 mV of hyperpolarization after correction for probe roll-off characteristics. Approximately 5% of closed time events appeared to be "nachschlags", i.e. were shorter than would be predicted by an exponential distribution of closed times which depended only on opening frequency and mean open lifetimes. Supported by NS 14920.



211.6 CHARACTERIZATION OF VOLTAGE- AND ION-DEPENDENT CONDUCTANCES IN VERTEBRATE HAIR CELLS. R. S. Lewis* (SPON: A. J. Hudspeth), Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Sound or acceleration evokes a receptor potential in vertebrate hair cells which modulates the release of transmitter onto auditory or vestibular nerve fibers. This receptor potential is a composite of the effects of several ionic currents. The transduction current, produced through deflection of the hair bundle, changes the cell's membrane potential and thereby activates voltage-dependent conductances. We have used whole-cell and excised-patch voltage-clamp techniques to identify and characterize these conductances in solitary saccular hair cells of the bullfrog, Rana catesbeiana.

Hair cells are isolated from the sacculus by treatment with 0.5 mg/ml papain followed by gentle scraping. Single cells are then sucked onto the end of a heat-polished pipette, forming a 1-20 gigohm seal with the glass. The pipette contains (in mM) 130 K, 120 aspartate, 2 Mg, 3 glucose, 2 EGTA, 5 Cl, and 5 HEPES (pH 7.2), with 0.1 μM free Ca. 130 mM Cs is substituted for K to isolate the Ca current. The cells are ruptured by applying stronger suction and are voltage-clamped and internally dialyzed through the pipette. A continuous-stream external perfusion system allows rapid and reversible changes in the extracellular milieu during recordings of ionic currents from an isolated cell.

Three ionic conductances which are activated by depolarization have been found using these techniques. An inward current, carried by Ca, Sr, or Ba, can be blocked with external Mg or Ni. This Ca current is activated at above -50 mV and has an activation τ of .4 msec at -20 mV and 22°C. Since it does not inactivate within at least 100 msec, it may underlie tonic transmitter release at the synapse onto auditory nerve fibers. Two outward K currents are present, both of which can be blocked by replacement of internal K with Cs, but which differ in their pharmacological sensitivities and kinetics. A non-inactivating, Ca-activated K current turns on at potentials above -50 mV and reaches a plateau value in 3-5 msec at -20 mV and 22°C. It is blocked by external tetraethylammonium (TEA; $K_{0.5}$=1 mM) but is relatively insensitive to external or internal 4-aminopyridine (4-AP). This current is also eliminated by substituting Mg, Ni, or Ba for external Ca or by removing all external Ca without substitution. The steady-state I-V curve for the Ca-activated K current peaks at a voltage dependent on the extracellular Ca concentration. In addition, there is evidence for an A-type K conductance in the hair cell. It shows voltage- and time-dependent activation and inactivation similar to that of previously-described A currents. It is relatively insensitive to external TEA but is partially blocked in a voltage-dependent manner by 10 mM 4-AP externally. The contributions of these ionic currents to the receptor potential of the hair cell will be discussed. (Supported by NIH grants NS-13154 and GM-07737.)

211.7 INWARD CALCIUM CURRENTS IN VOLTAGE-CLAMPED HIPPOCAMPAL PYRAMIDAL CELLS. W.H. Griffith and D.A. Brown*. Dept. of Pharmacology, School of Pharmacy, University of London, London, U.K.

We have previously described, in hippocampal cells, a slow Ca dependent outward potassium current (Brown, Griffith & Halliwell, 1982. J. Physiol., 324, 63P). We now present additional information on the calcium currents in these neurons.

Transverse slices (500 μm) from guinea-pig hippocampus were maintained at room temperature (23-26°C) for in vitro recording. The slices were held submerged and continually perfused with Krebs solution containing 3 mM KCl and 2.5 mM $CaCl_2$. Tetrodotoxin (0.5 μM) and tetraethylammonium (10 mM) were also added to block the Na^+ current and slow K^+ current, respectively. CA3 (n = 30) and CA1 (n = 29) cells were voltage clamped with a single KCl-filled microelectrode using a Dagan switch-clamp device. These neurons had a mean resting potential of 68 ± 1 mV and input resistance greater than 50 MΩ.

There appeared to be two types of I_{Ca} generated by depolarizing commands from about -50 mV: a slow, time and voltage dependent graded current similar to that reported by Johnston, Hablitz & Wilson (1980, Nature 286, 391); and a faster "spike-like" current. The two currents could be recorded separately in different cells or were co-existent in the same cell, and were observed in both CA1 and CA3 cells. As evidence for Ca-dependence, both currents were (a) eliminated on removing external Ca or adding Cd (0.1 mM), Mn (5 mM) or verapamil (100 μM), and (b) enhanced by adding 1-2 mM Ba. The fast current was suppressed and the slow current enhanced at holding potentials positive to -40 mV. Under these conditions the slow current generated by 10-30 mV depolarizing commands peaked after 0.2 - 0.3s.

Fast inward tail-currents recorded on repolarizing to E_K (to eliminate contaminating outward current) suggested that the slow I_{Ca} inactivated very slowly and incompletely after 0.7 - 1s. In accordance with this, the "steady-state" I/V curve in TEA showed inward rectification positive to -35 mV. The fast current could not be clamped adequately for kinetic measurements, and may represent a dendritic Ca-spike process. The slow current was most prominant in CA3 cells and was recorded in some cells showing no Ca-spike, and therefore may be involved in processes other than spike generation.

Supported by the U.K. Medical Research Council.

211.8 ACTION POTENTIAL REPOLARIZATION INVOLVES A FAST, CALCIUM-SENSITIVE POTASSIUM CURRENT IN A VERTEBRATE NEURON. A. B. MacDermott and F. F. Weight. Lab. Preclinical Studies, NIAAA, Rockville, Md.

Action potential repolarization in neurons usually involves a voltage-activated K^+ current which has rapid activation kinetics. In addition, there is a slowly activating Ca^{2+}-dependent K^+ current which is the primary hyperpolarizing current underlying the late, slow portion of the after-hyperpolarization following an action potential. We have studied electrical membrane activity in sympathetic neurons of bullfrog using intracellular recording and two electrode voltage clamp methods. Our results indicate that a fast, Ca^{2+}-sensitive K^+ current is primarily responsible for repolarization of the action potential. The large (>50 u) B cells in the IX and X sympathetic ganglion of the bullfrog were selected for impalement in these experiments. The preparation was continuously superfused with a normal, HEPES-buffered Ringer (pH 7.3) or a Ringer containing one of the following additional divalent cations: 2.5-5 mM Mn^{2+}; 8-10 mM Mg^{2+}; 0.5 mM Cd^{2+}; 3-5 mM Co^{2+}; or 8 mM Mg^{2+} and 0 Ca^{2+}. Under voltage clamp conditions, a fast outward current was activated by a voltage step from resting membrane potential (-50 to -60 mV) to -20 mV and more positive. The amplitude of the fast outward current was not only dependent on the activation voltage, it was also dependent on holding potential; the more negative the holding potential, the larger the response at a fixed activation potential. With the addition of a Ca^{2+}-blocking divalent cation, the fast outward current was significantly reduced. The remaining, fast outward current was 1) only activated from holding potentials negative to -70 mV and 2) apparently not Ca^{2+} sensitive. Both fast currents were blocked by 3-5 mM Ba^{2+} or 10 mM TEA. The amplitude of the fast outward current in normal Ringer decreased with the addition of high extracellular K^+ (10 mM), and the value of the extrapolated reversal potential of the tail current was more positive, suggesting that the fast outward current is carried by K^+ ions. The same conditions which blocked the fast K^+ current also significantly slowed the rate of repolarization of the action potential. The rapid activation of the fast, Ca^{2+}-sensitive K^+ current from resting membrane potential and the similar sensitivity of this current and the action potential repolarization to divalent cation blockade, suggest that action potential repolarization involves the fast Ca^{2+}-sensitive K^+ current. Since voltage-activated Ca^{2+} entry into the soma occurs during spike activity, the fast Ca^{2+}-sensitive K^+ current may act to rapidly repolarize the membrane thus closing voltage-activated Ca^{2+} channels, effectively inhibiting further Ca^{2+} entry.

211.9 IDENTIFICATION BY SINGLE CHANNEL RECORDING OF THE 5-HT-SENSITIVE K^+ CHANNEL RESPONSIBLE FOR SLOW EPSP IN APLYSIA SENSORY NEURONS. J. S. Camardo*, S. Siegelbaum*, and E. R. Kandel. Center for Neurobiology and Behavior, Depts. of Physiol. and Psychiatry, Columbia Univ., P & S, and N. Y. State Psychiatric Institute, New York, N.Y. 10032.

Serotonin induces a slow EPSP in the sensory neurons of Aplysia by decreasing a K^+ current (Klein and Kandel, PNAS, 77:6912, 1980). The modulation of this K^+ current facilitates transmitter release by delaying repolarization of the action potential in the presynaptic terminal, thereby prolonging influx of Ca^{++}. Serotonin produces this action by modulating a novel cAMP-dependent K^+ current unrelated to I_K, I_A, I_C and M current (Camardo et al., Soc. Neurosci. Abstr. 7:836, 1981). Using single channel methods, we have now identified a specific K^+ channel in these cells. The characteristics of this channel correspond to those of the 5-HT-sensitive current observed under voltage clamp.

Single channel records were obtained from Aplysia sensory neurons in abdominal ganglia exposed to 0.2% trypsin for 20 minutes. Using fire-polished patch electrodes, seal resistance of greater than 10 giga-ohms could be obtained with patches of membrane on the intact cell. The resistance was monitored with an intracellular electrode. Single channel outward K^+ current was observed under conditions of maintained depolarization of the membrane patch, which inactivates I_K and I_A. The K^+ channel has a conductance of 55 ± 6 pico-siemens at 0 mV. It is open at the resting membrane potential, shows moderate voltage dependence, and does not inactivate with maintained depolarization. Application of 5-HT to an area of the cell at a distance from the patch electrode increases the input resistance of the cell, and closes the channels under the patch. Channels which remain open, or re-open in the presence of 5-HT, show no change in conductance or reversal potential. Since the giga-ohm seal prevents access of 5-HT to the membrane directly under the patch electrode, modulation of the channels in the patch can be explained if a second messenger (cAMP) mediates the action of 5-HT in these cells. This idea was further supported by the intracellular injection of cAMP which reduced channel opening. Channel opening and current flow through these channels persist when the patch is isolated from the cell and its inside surface is exposed to a solution containing only K^+ as the cation, and a Ca^{++} concentration as low as 10^{-8}M.

We conclude that closing of this K^+ channel can account for the action of 5-HT on the action potential in the unclamped cell, and on the K^+ current under voltage clamp conditions. Its modest voltage sensitivity and failure to inactivate distinguish it from the early K^+ and delayed K^+ currents, and its persistent opening in the presence of 10^{-8}M Ca^{++} distinguishes it from the Ca^{++}-dependent K^+ current. The action of 5-HT on this current can be simulated by cAMP, suggesting that closure of the channel involves phosphorylation of the channel itself or of an associated protein by a cAMP-dependent protein kinase.

211.11 GENETIC CONTROL OF POTASSIUM CURRENTS IN DROSOPHILA. B. Ganetzky* and C.-F. Wu. Lab of Genetics, Univ. of Wisconsin, Madison, WI 53706 and Dept. of Zoology., Univ. of Iowa, Iowa City, IA 52242.

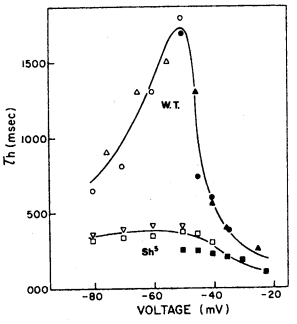
Previously we reported that eag (ether à go-go) and Sh (Shaker) two mutations that alter nerve membrane excitability and neuromuscular transmission displayed synergistic effects when combined in double mutants, suggesting the possibility of a functional relationship between the two genes (Ganetzky et al., Soc. Neurosci. Abstr., 7:543, 1981). These mutants have now been studied further by voltage clamp analysis of the larval body wall muscles. Sh mutants were found to reduce or eliminate the fast transient potassium current (I_A) but not the delayed rectification potassium current (I_K), consistent with voltage clamp studies in developing adult flight muscles (Salkoff and Wyman, 1981). However eag preferentially alters I_K. This result is further supported by observations of muscle membrane potentials under current clamp conditions. eag muscles produce regenerative Ca potentials in response to injection of depolarizing current. In normal muscles, delayed rectification prevents initiation of Ca potentials.

The extremely prolonged release of neurotransmitter at the larval neuromuscular junction which has been reported for the Sh^{tko120} strain is similar to that observed in eag Sh double mutants. Genetic and electrophysiological analysis of this strain demonstrated that it does, in fact, carry an additional mutation which is a new eag allele. This allele has a similar but less extreme physiological defect as the original eag allele. In all cases now examined, eag, Sh, and eag Sh double mutants are readily distinguished from each other by their neurophysiological phenotypes.

Pharmacological experiments supply additional evidence concerning the defect in eag and Sh. Neuromuscular transmission in normal larvae treated with 4-AP mimics that of Sh mutants. The phenotype of the eag Sh double mutant can be mimicked by TEA, but not by 4-AP even in high doses (Jan et al., Proc. R. Soc. Lond. B. 198:87, 1977). However, neuromuscular transmission in eag larvae treated even with low doses of 4-AP does resemble that of the double mutant. Results of these experiments indicate that the eag and Sh genes affect separate components regulating potassium currents in both nerve and muscle of Drosophila.
Supported by NIH grants NS 15390, NS 15350 and NS 15797 and a grant from Chicago Community Trust/Searle Scholars Program.

211.10 MUTANT DROSOPHILA POTASSIUM CHANNELS: GREATLY REDUCED VOLTAGE SENSITIVITY OF INACTIVATION RATE, Lawrence Salkoff. Dept. of Biol., Yale Univ., Box 6666, New Haven, CT. 06511.

Ion channels may be voltage sensitive with regard to the following properties: rate and amount of activation, rate of inactivation, and rate of recovery from inactivation. For normal wild-type Drosophila A-current channels the τ_h curve which describes the relation between the rate of inactivation (or recovery from inactivation) and voltage, is a steep bell-shaped relation (see fig. below). In contrast, the shape of the τ_h curve for mutant Sh^5 channels is profoundly flattened showing that, for these properties, the Sh^5 channels are far more indifferent to voltage. Although the Sh^5 mutation greatly reduces the voltage sensitivity of these properties, other voltage sensitive properties like the rate and amount of activation at different voltages, are not altered by this mutation. This seems to suggest that the mechanisms of activation and inactivation are functionally independent.



filled symbols: inactivation
open symbols: recovery

In other animal species the τ_h curve may be either steeply bell-shaped as in Helix (Neher, 1971, J. Gen. Physiol. 58:36) and Renilla (Hagiwara, et al., 1981, J. Physiol. 318:123), or flat as in Anisodoris (Connor & Stevens, 1971, J. Physiol. 213:21, and C.F. Stevens, personal communication). Thus, voltage sensitivity of inactivation time course and recovery is not a general property of A-current channels.

The Sh^5 mutation apparently eliminates the voltage sensitive process in inactivation. The fact that after this elimination, inactivation and recovery are more rapid (Salkoff & Wyman, 1981, Nature 293:228) suggests that the voltage-sensitive component retards the changes in state responsible for inactivation and recovery, rather than being the motive force for them.

212.1 RAPID CORRECTIONS OF POINTING MOVEMENTS OCCASIONED BY CHANGES IN TARGET LOCATION. J. F. Soechting and F. Lacquaniti*. Lab. Neurophysiology, Univ. Minnesota Medical School, Minneapolis, MN 55455.

The motor task studied required the production of a compound arm movement in the sagittal plane, involving motion at the shoulder and elbow joints. Subjects were asked to point to a target upon presentation of a tone. In about 50% of the trials, target location, which was indicated by a light rectangle displayed on a video screen, was displaced suddenly after the movement had been initiated, thus requiring a rapid modification of the trajectory of the arm movement. Elbow and shoulder angular displacement were recorded, as was EMG activity of biceps, triceps and deltoid muscles and net torque acting at the shoulder and elbow joints was calculated. It was found that the reaction time to modify the trajectory was about the same as that for movement initiation (about 100 ms). Furthermore, rapid modification of the trajectory was produced in a very stereotyped manner, which can be summarized as involving coordinated shoulder and elbow motion achieved by means of a reduction of the number of degrees of freedom of the movement. This task simplification took two forms. First, angular acceleration at the shoulder and elbow were linearly related during the deceleratory phase of movements involving a correction of the trajectory, such that the target was approached along the same path as in direct movements to the target. Secondly, when the correction demanded an increase or decrease in flexor torque at both shoulder and elbow, EMG activity in anterior deltoid and biceps both increased or decreased abruptly and simultaneously. However, when a correction called for increases in shoulder flexor and elbow extensor torque. triceps and deltoid were activated sequentially, It is concluded that rapid corrections of compound arm movements involve muscle synergies which express themselves as stereotyped patterns of activity in shoulder and elbow muscles.

212.2 PRE-MOVEMENT SILENCE IN AGONIST MUSCLES PRECEDING MAXIMUM POWER ARM MOVEMENTS. J.A. Mortimer and P. Eisenberg*. Geriat. Res. Educ. Clin. Ctr., V.A. Medical Center, Minneapolis, MN 55417, and Dept. Neurol., University of Minnesota, Minneapolis, MN 55455.

Pre-movement silence (PMS) was observed (Yabe, 1976) in agonist muscles preceding the initial burst of EMG discharge when subjects performed fast movements. In the present experiments, the occurrence and function of PMS were studied in subjects performing a maximum power flexion movement of the arm.

The right forearm of seated subjects was strapped into a lightweight arm support that could be rotated about the elbow in a horizontal plane. A torque motor provided a constant 3 Nm load tending to extend the arm as subjects maintained an initial 90° elbow angle. Subjects were instructed to respond to a tone stimulus with a maximum power arm flexion movement that was arrested by a foam cushion. Subjects were to respond either (a) as soon as possible after hearing the tone (RT) or (b) any time within 1 second of hearing the tone (non-RT). Surface EMG's were recorded from biceps, brachioradialis, lateral triceps and pectoralis muscles on the right side.

PMS occurred on some trials in all 5 subjects tested, and in one subject on over 95% of the trials under the non-RT condition. PMS durations ranged from 20 to 150 ms., and its latency following tone onset was as brief as 55 ms. (on RT trials). In nearly all cases, PMS was the first EMG change observed, preceding increases in the activity of agonist, antagonist and postural-stabilizing muscles. PMS was most clearly apparent on non-RT trials where a maximum power movement was required. PMS was absent when (a) the movement to be performed involved slow or moderate rates of force development or (b) when subjects responded at a very short latency following the tone. Longer PMS duration was significantly correlated with both longer reaction times to movement initiation and higher peak accelerations of the subsequent movement. In many instances, PMS was observed to occur in several synergistic muscles (biceps, brachioradialis, pectoralis). However, these decreases in motor activity were not necessarily simultaneous.

The findings suggest that PMS may play an important role in synchronizing motoneuron discharge during the initial agonist burst, leading to a more rapid development of force. The fact that longer duration PMS is associated with higher movement acceleration may be related to the increased percentage of motoneurons that become non-refractory as the PMS duration increases. Although the mechanism of PMS is unclear, the present results demonstrate that reciprocal inhibition is not involved.

Supported by the Veterans Administration.

Yabe, K., J. Appl. Physiol. 41:470-473, 1976.

212.3 CENTRAL PROGRAMMES FOR HUMAN BALLISTIC MOVEMENTS. B. T. Shahani and R.R. Young. Clinical Neurophysiology Laboratory, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

We have previously demonstrated that the central programmes subserving fast voluntary flexion and extension movements of human limbs during a visual matching task produce a triphasic EMG pattern (two bursts of EMG acitivity Ag 1 and Ag 2 in the agonist with a silent period Ag 1 -Ag 2 interposed between them and an EMG burst in the antagonist An 1) when the angular velocity exceeds 125°/sec. (Shahani and Young, 1980) The present study was undertaken to determine whether or not the centrally programmed triphasic EMG pattern, which is responsible for an accurate single ballistic movement, is also present during repetitive movements performed at the rate of 1 to 5 Hz. Using the same experimental paradigm, 5 health subjects, 22 to 37 years old, were asked to match the target by producing voluntary flexion or extension at the elbow and/or wrist joint. During a single ballistic movement and repetitive movements at 1 and 2 Hz a well defined triphasic EMG pattern was present in all subjects. This pattern changed to a single burst pattern during each flexion and extension movement with no evidence of Ag 2 or An 1 when the rate was increased to 3 Hz (or higher) at which time the electrical silence between the EMG bursts was significantly longer than the Ag 1 - Ag 2 interval in the same subject. Sometimes a triphasic pattern was seen during the initial movement (flexion or extension) followed by a single burst EMG pattern during subsequent repetitive activity at 3, 4 and 5 Hz. It is concluded that there are different types of central programmes for specific motor commands which utilize different strategies for bringing distal parts of the limb to the target with speed and precision. Furthermore, it is possible that the 3-7 Hz tremor-at-rest of Parkinson's Disease is related to the centrally programmed single burst EMG activity seen during repetitive movements rather than due to release of the triphasic pattern seen during a single ballistic movement, as suggested by some investigators.

212.4 AGONIST AND ANTAGONIST MUSCLE INTERACTION IN THE 8-12 HZ COMPONENT OF PHYSIOLOGIC TREMOR. R.J. Elble. Dept. of Medicine, Southern Illinois Univ. Sch. of Med., Springfield, IL 62708.

Physiologic tremor is comprised of two distinct component tremors. The most prominent component is the oscillation that any limb exhibits as a result of its underdamped mechanical characteristics, is present in all persons, and has a frequency that is inversely proportional to the square root of the limb mass. When this tremor becomes large, as during fatigue, reflexly evoked bursts in the agonist muscle EMG can be recorded (Stiles, R.N., J. Neurophysiol. 44: 40-59, 1980). Although the behavior of the antagonist muscles during this mechanical-reflex tremor has never been adequately studied, the work of Young and Hagbarth suggests that the agonist and antagonist tremor bursts should be 180 degrees out of phase (J. Neurol. Neurosurg. Psych. 43: 248-256, 1980). The second component of physiologic tremor has a frequency of 8-12 Hz. Fatigue and large external mass loads produce a slight increase (1-2 Hz) in the mean frequency of this component while these same maneuvers consistently reduce the mean frequency of the mechanical-reflex component (Elble, R.J. and Randall, J.E., Electroenceph. Clin. Neurophysiol. 44: 72-82, 1978). To further evaluate the 8-12 Hz component of physiologic tremor, surface EMGs of the extensor digitorum communis, extensor carpi radialis longus, and flexors carpi radialis and ulnaris were recorded simultaneously with tremor of the horizontally extended hand. The forearm was fully pronated and supported to the wrist. A piezoresistive accelerometer (5 gm mass) was taped to the dorsum of the hand. EMGs were full-wave rectified and lowpass filtered (bandpass 0-30 Hz; no phase shift) on a digital computer and were then analyzed using auto- and cross-spectral analysis. Using the protocol described in the above paper by Elble and Randall, the mechanical-reflex and 8-12 Hz tremor components were separated by applying 450-1000 gm loads to the extended hand. All ten subjects had a prominent mechanical-reflex component while only five subjects had a readily demonstrable 8-12 Hz component. The 8-12 Hz tremor was associated with prominent synchronous bursting in the agonist and antagonist EMGs. The synchrony of the agonist and antagonist tremor bursts was evident both by visual inspection and by rigorous cross-spectral analysis. The mechanical-reflex component was associated with minimal agonist and no demonstrable antagonist tremor-related activity. The synchronous agonist-antagonist 8-12 Hz tremor pattern is difficult to explain on the basis of an oscillating stretch reflex since reciprocal inhibition would be defied. This synchronous EMG pattern would appear to favor a central oscillator mechanism for this tremor component.

212.5 VARIABILITY OF FAST AND ACCURATE ARM FLEXION MOVEMENTS OF MONKEYS. D. Flament*, J. Hore and T. Vilis (SPON: T. Feasby). Department of Physiology, Univ. Western Ontario, London, Ont., Canada N6A 5C1.

When analyzing fast and accurate elbow flexions made by 5 Cebus monkeys, we noticed that considerable variability occurred in the duration and magnitude of the acceleration and deceleration phases of individual movements of the same amplitude and the same velocity. We therefore examined the question of whether this variability was totally random, or whether there was some causal relationship between the variability in the acceleration phase and that in the deceleration phase.

When examining durations we found that, as in the human arm movement studies of Lestienne 1979, the duration of acceleration was inversely related to peak velocity for a given target displacement. Surprisingly we also found that the same inverse relationship held for the duration of deceleration. Thus large velocity movements had shorter decelerations than low velocity movements of the same amplitude. Movements of smaller amplitudes also followed these inverse relationships but with a smaller velocity for a given acceleration or deceleration duration.

We then extended the work of Lestienne by examining the trial-to-trial variation in duration and magnitude of acceleration and deceleration for movements of the same velocity and the same target displacement. We found that 1) movements with a short acceleration had a longer deceleration than the mean for that velocity, and 2) movements with a large acceleration magnitude had a smaller deceleration magnitude than the mean for that velocity.

This analysis indicates that movements of a given velocity can have considerable scatter in both the magnitude and duration of the acceleration phase. However this scatter is compensated for in the deceleration phase so that, if the acceleration is of a short duration and large magnitude, the deceleration becomes of a long duration and small magnitude. This ensures that in spite of the variation in the acceleration phase, the total movement displacement is accurate. Thus the deceleration phase is not only a preprogrammed sub unit of the acceleration command but is capable of correcting errors in the acceleration command.

Lestienne, F. Exp. Brain Res. 35, 407-418, 1979.

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212.6 MATCHING OF MOVEMENTS MADE INDEPENDENTLY BY THE TWO ARMS IN NORMAL HUMANS. D. al-Senawi* and J. D. Cooke (SPON: J. D. Brown). Dept. of Physiology, Univ. of Western Ontario, London, Canada.

Although it is well known that most humans show a limb preference, it is also clear that we can carry out well co-ordinated movements with the two arms. In this study we present data showing that certain movement parameters are 'matched' when a subject makes simple movements independently with either arm. The movements studied were visual step-tracking movements about the elbow joint. During experiments the subject was seated comfortably with forearm positioned horizontally and supported at the elbow. The subject grasped a vertical handle at one end of a manipulandum. The other end of the manipulandum was pivoted just above the subject's elbow joint. The subjects performed alternate flexion/extension movements about the elbow while target and handle positions were displayed to them on an oscilloscope. No restrictions were placed on movement velocities or reaction times. In any one experimental session the subject would be asked to make a series of movements of five different amplitudes with one arm. The equipment would then be repositioned and the series repeated with the contralateral arm.

In all subjects tested (n=6), the relation between movement peak velocity and movement amplitude was linear ($r>0.9$). The slope of this relation was identical for movements made by the two arms. With intermittent testing, this relation was found to be invariant in each subject for periods of up to two months. Six subjects were asked to continuously wear a 1 lb weight strapped to the forearm of their left (non-dominant) arm for up to one week. Addition of the weight to the left arm produced an increase in the slope of the peak-velocity/amplitude relation in this arm. This was seen with or without the weight on the arm during testing. This change in slope was exactly matched in movements made independently with the right (non-loaded) arm. A similar matching of movements was seen following removal of the weight. It is suggested that this matching of movements by the two arms is a reflection of basic mechanisms of inter-limb co-ordination. In addition we suggest that matching of performance will occur following any unilateral disturbance of normal movement performance.

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212.7 POSITION - VELOCITY - TORQUE RELATIONS DURING HUMAN ARM MOVEMENTS. J. D. Cooke, Dept. of Physiology, University of Western Ontario, London, Canada.

Many recent studies have concentrated on the role of the mechanical properties of muscle in the maintenance of limb position. It has been suggested that at rest the oppositely directed spring-like forces generated by opposing muscles are in equilibrium. The experiments reported here are directed at the translation from one equilibrium position to another.

Studies were made on normal human subjects performing a visual step tracking movement. Subjects were seated and grasped with semi-pronated wrist a vertical handle attached to a manipulandum. The manipulandum could be rotated in the horizontal plane, the subject's elbow being positioned under the pivot point of the manipulandum. Subjects made alternate flexion/extension movements about the elbow between two target positions. Target positions as well as handle position were displayed to the subject on an oscilloscope. The net torque exerted by the subject was measured with a balanced strain gauge bridge mounted 5 cm from pivot point of the handle. Forces could be applied to the handle by a torque motor.

Curves of torque vs position during movement were linear over the greater part of the movement. The slope of these curves represent the angular stiffness of the system being moved (arm plus manipulandum plus motor). Movements of different amplitudes were made with constant angular stiffness, the equilibrium angle being changed. Movements of constant amplitude but varying velocities were accompanied by changes in angular stiffness: the more rapid the movement the higher the angular stiffness.

It is suggested that trajectories of the whole limb during movement against constant inertial load can be closely approximated by simple step changes in the properties of state of the limb musculature.

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212.8 EFFECTS OF MOVEMENT AMPLITUDE ON COMPONENTS OF THE INITIAL AGONIST BURST. Susan H. Brown* and J. D. Cooke (SPON: V. B. Brooks). Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada.

Recent investigations in this laboratory have shown that the initial agonist burst of the triphasic EMG pattern in humans is, in fact, composed of two functionally independent components. These components are not restricted to fast movements but are present in slower, more accurate movements as well. Since it has been shown that the magnitude of the initial agonist burst is dependent upon movement amplitude, experiments were performed to determine the effects of movement amplitude on each component. In addition, preliminary experiments were performed to examine the pattern of motor unit activity associated with the initial agonist burst.

Subjects performed a series of step-tracking flexion movements ranging from 10 to 50 degrees about the right elbow. Emphasis was placed on either speed or accuracy, depending upon the given trial. Surface EMG activity was recorded from biceps and triceps muscles. For the motor unit studies, fine-wire electrodes were inserted into the biceps muscle.

In all subjects both EMG components increased in magnitude with increasing movement amplitude. In general, the first component was of slightly greater magnitude than the second component. Both components changed magnitude in parallel, i.e. proportional increases were observed in both components as movement amplitude increased. This was true for both instructions. The slope of the relation between EMG magnitude and movement amplitude was instruction-dependent: fast movements producing a greater slope than accurate movements. In addition to the parallel changes which occurred in both components, peak velocity also appeared to be closely coupled to changes in component magnitude.

Both components were clearly distinguishable from records of motor unit activity associated with flexion movements. Small units usually showed two bursts of activity during the time of the initial burst. As movement velocity increased, larger units fired only during the first component or, at the highest velocities, during both components. In movements characterized by decreased acceleration, large units were active only during the second component.

The results suggest that, for simple tracking tasks, both components are modulated in parallel and that the recruitment of motor units during one or both components is dependent upon movement strategy.

(Supported by the Medical Research Council of Canada (MT-6699)).

212.9 AMPLITUDE-DURATION-VELOCITY RELATIONSHIPS FOR HEAD, JAW, AND FINGER MOVEMENTS MADE BY HUMAN SUBJECTS. E.S. Luschei*, A.F. Fuchs, M.D. Binder and M.E. Anderson. Depts of Physiol. and Biophys. and Rehab. Med. and Regional Primate Research Cent., Univ. of Washington, Seattle, WA 98195.

The amplitude of a movement could be increased by recruiting additional motor units, increasing the firing frequency of individual motor units or prolonging the time period during which the motor units discharge. In the case of saccadic eye movements, there is a clear positive correlation between their amplitude and duration. A different strategy has been reported, however, for rapid finger movements, in which the duration appears nearly constant over a considerable range of movement amplitudes. Movement amplitude is increased in these movements by a change in velocity. To determine whether distinct strategies are, indeed, employed by the nervous system to modulate different movements, we have examined the amplitude-duration-velocity relationships and the underlying EMG activity for rapid, target-directed movements made by three body parts: the jaw, the head, and the index finger.

Data were collected from three subjects without prior training in the tasks. Movement amplitude, velocity and duration were measured with a phototransducer that produced a voltage proportional to the position of a light rigidly fixed to the moving segment.

The most striking aspect of the data is the variability in strategies used by different subjects. For jaw opening movements, for example, correlations between movement amplitude and duration ranged from .19 to .94. The subject with a high correlation and steep slope in the amplitude-duration relationship showed relatively little scaling of peak velocity, whereas the subject who had little or no amplitude-duration relationship clearly increased peak velocities with movement amplitude. Head movements also showed marked variations between subjects. One subject primarily varied peak velocity and EMG amplitude in both agonist and antagonist muscles but had no significant change in movement duration. A second subject had a high correlation between amplitude and duration, some scaling of peak velocity and EMG amplitude in the agonist, and a decrease in antagonist activity with larger movements. The third subject had a low correlation between amplitude and duration, high peak velocities for all amplitudes of movement, but an increase in antagonist activity and a second burst of agonist activity as movement amplitude increased. Finger movement showed considerable scatter in points relating amplitude and duration for all three subjects, but peak velocity clearly covaried with movement amplitude.

What is clear from these data is that no single neural strategy is used by all subjects to scale the amplitude of rapid movements of a single body segment.

212.10 **CONTRIBUTION OF PERIPHERAL FEEDBACK TO THE CONTROL OF A RAPID FLEXION MOVEMENT OF THE FOREARM IN MAN.** R. Forget* and Y. Lamarre. Centre de recherche en sciences neurologiques. Fac. de méd., Univ. de Montréal, Montréal, Québec, Canada H3C 3J7

The electromyographic activity (EMG) of the biceps (Ag) and the triceps (Ant) was studied during a rapid flexion movement of the forearm at two amplitudes (40 and 90 degrees) in ten normal subjects and one patient with a polyneuropathy affecting selectively the peripheral sensory myelinated fibers. In response to a sound cue the subjects had to move from the departure zone to a fixed target zone displayed on a cathode ray monitor. When the movement reached a peak velocity (Vmax) of about 200°/s, all normal subjects showed a triphasic EMG pattern characterized by two bursts in the agonist (Ag_1 and Ag_2) and a burst in the antagonist (Ant) occurring between Ag_1 and Ag_2. Tonic EMG activity of the Ant decreased 20 to 40 ms before the onset of Ag_1. The maximum Ant activity occurred at Vmax for the 40° movements and 30 ms after Vmax for the 90° movements. The time interval between maximum Ag_1 activity and peak acccelération (Amax) was the same as the interval between maximum Ant activity and peak deceleration (Dmax). The integrated EMG of Ag_1 and Ant showed a strong correlation with Amax and Dmax.

In the patient both Ag_1 and Ag_2 were present; tonic EMG activity of the triceps decreased before the onset of Ag_1 as in the normal subjects but there was no burst in the Ant during the movement. The patient had difficulty to perform the task, mostly the fast 90° movements which she correctly made in only 1/3 of the trials. For the 40° movements Dmax was well correlated with Amax as in the normal subjects, however this correlation was not found for the 90° movements. These data show that the antagonist burst of the triphasic pattern is important for rapid deceleration of the limb and that peripheral feedback contributes to the generation of this burst.

(This work was supported by the Canadian Medical Research Council).

212.11 HUMAN STRETCH SERVO RESPONSES TO LOAD DISTURBANCES IN FATIGUED MUSCLE. W.G. Darling*, K.C. Hayes* (SPON: C. MacKenzie) Dept. of Physiology, Univ. of Western Ontario, London, Ontario N6G 1H1

The human triceps brachii muscle load-compensating responses to torque motor perturbations have been investigated in sixteen normal subjects while performing submaximal fatiguing isometric contractions. The purpose of this study was to investigate the possibility of compensatory adjustments in the stretch servo under conditions of muscular fatigue i.e. the question of whether or not mechanical performance can be sustained in the face of a change in one of the internal state variables of the muscle. Subjects held low intensity isometric extension torques until they were unable to maintain a specified elbow-angle (90 deg.). They were instructed to resist two types of unpredictable perturbations (200 msec torque-pulses delivered through a manipulandum controlled by an Aeroflex TQ14DW-1F torque motor). These perturbations either stretched or shortened the contracted triceps muscle and subjects attempted to return their arm and manipulandum to the initial position as quickly as possible. M_1, M_2-M_3 and voluntary EMG responses were recorded from surface electrodes located over the lateral head of the triceps muscle. These signals were rectified, A/D converted at 1250 Hz, and averaged (Dagan 4800) prior to computer processing. The average amplitude of the EMG during the fatiguing contraction increased by 60 ± 27%. In the majority of subjects there was a prominent grouping of action potentials at 8-15 Hz (as revealed by demodulation and autocorrelation analysis) indicative of an enhanced physiological tremor. The net M_1 and M_2-M_3 responses to stretch increased progressively in 6 of the subjects throughout the fatiguing contraction. In 8 of the remaining 10 subjects there was evidence of an increase followed by a decrease in the reflex responses prior to terminating the contraction. No subjects presented a progressive decrement in stretch response amplitudes. In response to shortening perturbations, the net EMG activity during the M_2-M_3 interval (55-105 msec) progressively reduced throughout the fatiguing contraction to an overall decrement of 59%. This decrease was directly proportional to the increased amplitude of the background EMG activity which may be considered reflective of the internal loop gain of the system. Thirteen of the subjects showed this marked enhancement of the internal servo response under conditions of fatigue. No noticeable change in the mechanical performance was observed. These results confirm and extend previous reports of internal compensatory adjustments in the stretch servo offsetting the deleterious mechanical effect of muscular fatigue.

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212.12 THE ROLE OF CENTRALLY-GENERATED MOTOR COMMANDS AND SENSORY SIGNALS IN THE PERCEPTION OF MUSCULAR FORCE. L.A. Jones and I.W. Hunter. Psychology Dept. & Biomedical Engineering Unit, McGill University, Montreal, PQ Canada H3A 1B1.

It has been suggested by several investigators that there is a dissociation between estimates of effort and force made by human subjects, and that it is possible under some experimental conditions (e.g., during muscle tendon vibration) for an observer to monitor each of these "senses" independently. A sense of effort refers to signals generated within the central nervous system which are presumed to derive from the voluntary motor command sent to the muscle, while a sense of force or tension refers to the sensory representation of the force applied during a voluntary contraction and is based on the response of intramuscular receptors. The relative contribution of each of these two signals in estimating force was investigated in a series of experiments.

Isometric contractions of the elbow flexor muscles were maintained at different contraction levels (35%, 50%, 65% MVC) until endurance time. At 15 second intervals subjects estimated the force of the sustained contraction with a brief matching contraction of the contralateral muscle group in the unfatigued arm. Brachial biceps and triceps EMGs and the forces produced at the wrists were recorded from each arm.

Estimates of force increased throughout the maintained contraction, and the rate of increase paralleled the change in the biceps EMG in the fatiguing arm. At endurance time subjects over-estimated the force exerted by as much as 100%. However when subjects were instructed (after achieving a target force) to maintain an isometric contraction "so that it felt the same" for 2 minutes there was an exponential decrease in the force exerted which reached an asymptote at a force of 30% MVC. The brachial biceps EMG remained essentially constant for higher forces, but during the lower force contraction there was an increase in the EMG.

These results lend further support to the notion that the efferent command sent to the muscle or some corollary discharge is the preferred signal in judgments of force. However they also suggest that during muscular fatigue it is not possible for subjects to accurately estimate the force exerted by a muscle, and that under these conditions there is no dissociation between estimates of force and effort.

212.13 ATTAINING A LEARNED EQUILIBRIUM POINT AFTER A CHANGE IN SPATIAL TRAJECTORY. D. Larish* and S. Wallace* (SPON: C. Gisolfi). Motor Behavior Laboratory, Univ. of Iowa, Iowa City, Iowa 52242. The aim of the present study was to determine if a learned equilibrium point and muscle resting length could mediate accurate limb movement when a change in spatial trajectory forced a reorganization of an agonist-antagonist muscle relationship, while the equilibrium point itself remained constant. If a movement, the principle goal of which is to attain a predefined spatial target, is controlled primarily by muscle resting length, then a change in trajectory should not impair the limb's ability to satisfy the intended movement goal.

Experiments were conducted with normal human subjects performing rapid, horizontal flexion and extension movements about the elbow. In an initial experiment one group of subjects (N=12) learned the position of a 60° or 90° target via flexion movements, and then in a subsequent test phase were required to locate the target by making both flexion and extension movements. (Vision of the arm and target were occluded.) In a second group (N=12) the two targets were learned via extension movements, with the test phase consisting of flexion and extension reproduction. In the flexion training group, accuracy on flexion and extension trials was equivalent, however, in the extension training group accuracy on extension trials was better than on flexion trials. Moreover, this extension-flexion difference persisted even after added practice. In a final experiment, a within subjects design was used to reexamine performance in these two training groups. As before, when the learning phase consisted of extension movements, subsequent accuracy was greater for extension movements than flexion movements, and this difference again persisted after an extended practice period. Quite remarkably, when the learning phase consisted of flexion movements reproduction accuracy for flexion and extension movements was equivalent, and this pattern remained unchanged by an extended practice period.

The present findings suggest that the equilibrium point and muscle length can not always regulate the accurate attainment of a spatial target. From a theoretical point of view, these results are damaging to the equilibrium point notion. If the control of movements such as those used here are to be accounted for on the basis of muscle resting length, reproduction accuracy for flexion and extension movements should have been equivalent in both training groups, not just flexion training. Instead, there appears to be a certain degree of specificity in setting the length-tension relationships of the agonist-antagonist synergy. In some instances the CNS can extrapolate specifications of the equilibrium point outside the specific conditions under which it was established, yet in other instances, where the only difference is the initial learning situation, the CNS lacks this capability.

212.14 CONTROL OF RAPID BIMANUAL AIMING MOVEMENTS: THE EFFECT OF A MECHANICAL BLOCK. D.C. SHAPIRO* and C.B. WALTER* (SPON: G.P. Moore). Motor Control Lab., UCLA, Dept. of Kinesiology, Los Angeles, CA 90024.

Interlimb coordination was examined for a rapid bimanual task requiring subjects to simultaneously position right and left levers to targets. Previous literature suggested that the first 100 ms of rapid aiming tasks are programmed in advance (Wadman, W.J., et al., J. Hum. Mov. Stud., 5: 3-17, 1979). To examine this, a mechanical block was introduced to one limb and its effect was examined on the blocked limb as well as the contralateral limb.

The task involved horizontal elbow flexions of 200 ms to targets located 30° from each start position. Subjects practiced the task for 100 trials with vision of the targets and received movement time feedback following each response. After practice, 200 additional trials were performed of which 10% were blocked unexpectedly so that the left limb could not move. On each trial, EMG activity of the biceps brachii (agonist) and lateral triceps (antagonist) were recorded with surface electrodes from both limbs and displacements were indicated by potentiometers connected at the base of the levers.

Each flexion movement was initiated by a rapid rising agonist burst averaging 120 ms followed by a silent period. Perhaps a second agonist burst was not found because overshooting was rare (Ghez, C., Martin, J.H., Exp. Brain Res., 45: 115-125, 1982). Biphasic antagonist bursts were observed separated by 10 ms. The first burst was coactivated with the agonist burst (Lestienne, F., Exp. Brain Res., 35: 407-418, 1979) while the second burst occurred during the silent period. When comparing across limbs, the kinematic data as well as the EMG activity displayed similar characteristics. Comparison of the EMG activity of the blocked limb with the same limb when it was not blocked showed the first 120 ms of activity was maintained. The agonist burst, as well as the beginning of the first antagonist burst were identical across conditions, although no movement occurred in the blocked condition. These similar patterns preceded the time where displacement would normally begin. Two additional agonist bursts of progressively decreasing magnitude occurred in the blocked limb. The second agonist burst occurred on average 20 msec after movement displacement would normally have been initiated. Additional antagonist activity tended to be coactivated with the second agonist burst. Examination of the unblocked limb during blocked trials revealed no change in kinematic and EMG activity, and thus was unaffected by the contralateral block. Our data suggested that each limb may be independently controlled, and that adjustments made by the blocked limb may be due initially to reflex adjustments. (Supported by UCLA Academic Senate Grant).

212.15 EVIDENCE THAT ANTAGONIST MUSCLE ACTIVITY IS CENTRALLY GENERATED. D.S. Hoffman and P.L. Strick. V.A. Med. Ctr. and Depts. of Physiol. and Neurosurg., SUNY-Upstate Med. Ctr., Syracuse, NY 13210.

There is a well-defined pattern of muscle activity associated with ballistic movements to a target. Each movement is initiated by a burst of agonist muscle activity and decelerated by a burst of antagonist muscle activity. Whether the antagonist burst is generated by central programs or peripheral feedback is still a matter of controversy. These experiments were designed to provide new data on this issue.

Human subjects performed a visual tracking task that required ballistic wrist movements which ranged between 5 and 30 degrees of radial or ulnar deviation. On some trials subjects were instructed to move as fast as possible to the target and on others they were to move at slower speeds. Muscle activity was recorded from extensor carpi radialis longus and extensor carpi ulnaris.

When subjects attempted to move as fast as possible, they kept movement duration nearly constant even though movement amplitude was varied. Thus, larger amplitude movements were performed at greater peak velocities than smaller amplitude movements. The amplitude of the initial burst of agonist muscle activity was well-correlated with the magnitude of the peak velocity of each movement, and therefore, with movement amplitude.

A comparison of movements with different durations indicated that many small, quick movements were performed with the same initial velocity and the same amplitude of agonist muscle activity as large, slow movements. Thus, amplitude of the agonist burst remained well-correlated with velocity but could be dissociated from movement amplitude and duration. The initial antagonist bursts of the small, quick movements were larger than those of the large, slow movements. Therefore, differences in antagonist muscle activity led to the differences in movement amplitude and duration.

Our observations indicate that the antagonist burst exerts a powerful influence over movement amplitude and duration. Furthermore, the amplitude of the antagonist burst can be varied independently of initial movement parameters and the amplitude of the agonist burst. These findings provide additional evidence that antagonist muscle activity is centrally generated. Supported by funds from the V.A. Medical Research Service.

212.16 MUSCLE MECHANICAL PROPERTIES MEASURED DURING VOLUNTARY MOVEMENT. Thomas Zeffiro. Dept. of Psychol., M I T, Cambridge, MA 02139.

Previous studies of primate limb movements have utilized kinematic information and electromyographic data to infer the processes by which muscle mechanical properties are controlled during movement. A direct test of the resultant hypotheses was not possible without methods to measure muscle force in behaving animals. We have developed a technique which, combined with appropriate behavioral paradigms, provides new information about the relationships among muscle length, velocity, and force during movement. These results serve to bridge the gap between studies of isolated muscle properties and theories of movement.

We developed a force transducer both to measure muscle force and control the muscle length/joint angle relationship. Its characteristics include: (1) mounting on the ulna, (2) connection to the triceps tendon, (3) sensitivity to muscle forces in the physiological range, (4) calibration of the load sensing element without surgical intervention, (5) preservation of the physiological moment arm of the triceps, and (6) control of muscle rest length over a 17 mm range.

Rhesus monkeys were seated with one forearm attached to a manipulandum which allowed movements about the elbow joint in the horizontal plane. The animals tracked moving targets across a perimeter arc with elbow flexion/extension movements. Elbow angle, elbow velocity, elbow torque, triceps force, and EMG of the muscles acting about the elbow joint were measured. Triceps length was derived from elbow angle.

In a step tracking paradigm the target made rapid jumps to a final position while the torque motor provided constant loads about the elbow joint. After the arm had reached its final position, triceps force, triceps EMG and elbow angle were sampled. A family of static length-tension curves for the triceps muscle was obtained. The tricep's static stiffness (derived by computing the curve's slope at various lengths) increased with muscle length at low activation levels, but decreased with muscle length at higher levels of activation.

In a continuous tracking paradigm the target moved at constant velocity while constant loads were applied. Force, EMG and elbow angle were sampled at various tracking velocities and a family of force-velocity relations was derived. The muscle's dynamic stiffness was derived by computing dF/dL at various shortening and lengthening velocities. Although little velocity dependence was seen at low levels of activation, force declined dramatically during shortening contractions at higher activation levels. The magnitude of the force-velocity effect observed during these arm movements reveals the inadequacy of applying static approximations to movement dynamics. (Supported by NIH grants AM27610, EY02621 and NIGMS 5 T32 GM07484.)

212.17 SHORT-LATENCY LINKAGE BETWEEN HUMAN ELBOW ANTAGONISTS APPEARS REVERSIBLE THROUGH ALTERATION OF JOINT PRELOADING. R. W. Angel, C. C. Boylls, and M. R. Zomlefer. RERanD Center, VA Medical Center and Stanford University School of Medicine, Palo Alto, CA 94304.

The principle of reciprocal innervation would suggest that a human subject, instructed to maintain the position of a joint, would respond to a perturbation of that joint with activation of any "loaded" (stretched) muscles and inactivation of their "unloaded" counterparts. We tested this expectation by applying brief (20 ms) torque pulses which tended to flex or extend the elbow while the subject attempted to hold the joint at a 90 degree angle. The biceps (or triceps) was preloaded in one of two ways: (1) a constant force was applied by the torque motor, or (2) the subject contracted the antagonist muscle, thus requiring the agonist to produce the same level of electromyographic (EMG) activity as under condition 1. The direction of the torque pulse was varied randomly from trial to trial. Using averages of 11-16 trials in 4 subjects, we found that all the EMG responses in the 80 ms interval following the perturbation differed under the two conditions. Under condition 1, the paired muscles often responded together instead of reciprocally. Thus, an extension perturbation would elicit near-simultaneous contraction of both biceps (the stretched muscle) and triceps (the unloaded muscle). Under condition 2, the biceps showed an increase of activity and triceps showed a decrease, as predicted by the principle of reciprocal innervation. Our preliminary findings thus suggest that the reflex linkage between elbow antagonists may depend on the preloading conditions. If one muscle is preloaded by an external force, a sudden increase of this force may elicit a "paradoxical" contraction of the antagonist. If instead, preloading of the agonist is produced by contraction of the antagonist, the sudden increase of the external force may elicit reciprocal responses in the muscle pair. A reciprocal linkage of antagonists presumably effects alterations of net torque about a joint, while a covarying activation can be imagined to implement parametric changes in muscle dynamics (stiffness, damping, etc.). Why the reflex system should choose to support either the parametric or the torque-control strategy in any specific circumstance is a subject we presently are investigating.

212.18 EFFECT OF ISCHEMIC DEAFFERENTATION ON PATTERNS OF MUSCLE ACTIVITY DURING RAPID MOVEMENTS. Von Ayre Jennings* and Jerome N. Sanes. (SPON: R. P. Hammer, Jr.). Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205.

The electromyographic activity (EMG) associated with rapid voluntary limb movements has a distinctive triphasic pattern: an initial burst in the agonist (A1) is followed by a burst of antagonist activity (ANT) which in turn is followed by a second burst in the agonist (A2). A question of some interest has been the extent to which this EMG activity is generated by central commands or peripheral feedback occurring during movement. While it is clear that the occurrence of A1 is preprogrammed, it is equally certain that the duration and amplitude of this burst is influenced by sustained or transient modifications in kinesthetic input. A similar situation of ANT and A2 being mediated by both central commands and peripheral feedback may also exist. We investigated whether central commands contributed to ANT and A2 by examining EMG patterns in subjects who performed rapid voluntary movements with limbs that were functionally deafferented using the technique of ischemic blockade of sensory fibers.

Five subjects with their hands coupled to a servo-controlled torque motor performed rapid 20° wrist flexion movements. Surface EMG was recorded from the extensor and flexor carpi ulnaris muscles. The arm was reversibly deafferented below the elbow by wrapping a blood pressure cuff around the arm above the elbow and inflating the cuff to 180-200 mm of Hg for 20-40 min. The lower arm was determined to be functionally deafferented when light touch and joint sensation were absent and when ramp movements of the handle which passively flexed the wrist 20° in 100 ms failed to evoke a stretch reflex.

When subjects were ischemically deafferented the triphasic EMG pattern accompanying voluntary movement was preserved although the average amplitude of all three bursts was diminished. A varying amount of muscle weakness accompanied loss of peripheral sensation. In addition ANT and A2 were unusually small or even absent for slower movements that in the intact subject still showed the triphasic burst pattern. From these results we conclude that somatosensory information is not necessary for the production of the triphasic EMG pattern observed during rapid voluntary movements although such inputs may play an important role in determining both the amplitude and duration of the EMG bursts.

212.19 CHANGING GLOBUS PALLIDUS ACTIVITY AFFECTS SCALING BUT NOT SEQUENTIAL ORGANIZATION OF MUSCLE ACTIVITY. Fay B. Horak and Marjorie E. Anderson. Depts. of Physiology and Biophysics and Rehab. Med. and Regional Primate Research Center, Univ. of Wash., Seattle, WA 98195.

We have reported previously (Horak and Anderson, 1980) that both kainic acid-produced lesions and microstimulation in the globus pallidus of monkeys making rapid arm reaching movements in a forced reaction time task prolonged movement duration but did not delay movement initiation, measured as the reaction time. We now report the topographical organization of these effects.

Lesions produced the most profound and long-lasting increases in movement time of the contralateral arm when neurons were destroyed in the ventrolateral and caudal aspects of the internal (GPi), as well as external pallidal segment (GPe). Destruction of ventrolateral GPe, but not GPi neurons or of neurons in dorsomedial portions of GPe and GPi produced smaller and less persistent increases in movement time. In contrast, stimulus-induced increases in movement time were usually the result of stimulation at sites in GPe, dorsomedial GPi, or the putamen, and stimulation in ventrolateral GPi or in the region of the ansa lenticularis actually reduced movement times for the contralateral arm. Since stimulation at many sites could activate inhibitory striatopallidal fibers or GPe neurons that may inhibit an excitatory subthalamic input to GPi, we would suggest that contralateral arm movements are slowed when the output of the GPi neurons influencing these movements is reduced, either by an inhibitory process or by destroying the neurons, and that the arm movements are speeded when the relevant GPi neurons or their axons are excited by microstimulation.

Changes in movement duration produced by either kainic acid lesions or microstimulation were associated with a generalized change in the amplitude and rate of rise of EMG activity in all of the contralateral muscles studied. Ipsilateral EMG activity during performance of the same task was not affected by lesions or stimulation. Stimulation at different sites within GP, even at stimulus intensities as low as 25 uA, did not selectively affect the activity of the contralateral back, shoulder, elbow or wrist muscles. When movement speeds were changed by microstimulation, the sequential activation of different muscles and their rate of buildup in activity was similar to that for control (non-stimulation) movements of the same duration.

These experiments suggest a role for globus pallidus output in scaling the magnitude and buildup of EMG activity in different muscles, without affecting the initiation or the sequential organization of the programmed motor output. Supported by NIH grants NS15017, GM7108, & PRO-RR00166, & NIHR grant 16-P-56818.

212.20 CALLOSAL SECTION ABOLISHES BIMANUAL COORDINATION DEFICIT RESULTING FROM SUPPLEMENTARY MOTOR AREA LESION IN THE MONKEY. C. Brinkman* (SPON: R. Porter). Experimental Neurology Unit, The John Curtin School of Medical Research, Canberra, Australia 2601.

After unilateral ablation of the supplementary motor area (SMA) in the monkey, a transient forced grasping of the contralateral hand has been found (Smith et al, Brain Res. 222:395, 1981) as well as a transient, bilateral apraxic syndrome (Brinkman, Proc. Austr. Physiol. Pharmacol. Soc. 11:170P, 1980). The only lasting deficit after such a lesion, however, is one of bimanual coordination. Faced with a raised perspex platform which contains small holes in which currants have been placed, normal monkeys will push the bait out with the index finger of one hand while the other hand is cupped underneath to catch the falling bait. Monkeys with unilateral SMA lesions had great difficulty accomplishing this task. Analysis using high speed movie films showed that, after a unilateral SMA lesion instead of sharing the work load, both hands tended to be used in a similar fashion, e.g., both pushing the bait from either side of the platform in an animal with a lesion of SMA opposite the 'catching' hand (Brinkman, Neurosci. Lett. 27:267, 1981). This suggested that the intact SMA now influenced not only the ipsilateral primary motor area but also the contralateral one, presumably through the corpus callosum. Therefore, in two animals with unilateral lesions of SMA, the corpus callosum was divided and the monkeys retested on the bimanual coordination task. Immediately postoperatively, they showed mirror responses of the hands (but not necessarily at the same hole in the plate) which are seen also in normal split-brain monkeys (Brinkman and Kuijpers, Brain 96:653, 1973). However, within a week, these had disappeared and both animals now performed the task in a way indistinguishable from that of intact animals, and from their own performance before the SMA ablation. These findings support the idea that SMA is involved in motor programming, especially in bimanual coordination tasks; but also in the coordination of movements of a single extremity, since the transient apraxia seen in either hand after the initial SMA ablation returned after section of the corpus callosum. (Supported by grants from the National Health and Medical Research Council and from the Australian Research Grants Committee).

213.1 NEURAL EVENTS UNDERLYING ESCAPE SWIMMING BEHAVIOUR IN THE SQUAT LOBSTER, GALATHEA STRIGOSA (ANOMURA). K.T. Sillar* and W.J. Heitler. Gatty Marine Laboratory, The University of St. Andrews, St. Andrews, KY16 8LB, Scotland.

Investigations into the escape responses of crayfish have revealed three underlying neural subsystems. Two types of response are mediated by lateral and medial giant fibres for escape from caudal and rostral stimuli respectively, and the third, backward swimming involves many unidentified neurons and is poorly understood. All three result in an initial abdominal flexion projecting the animal away from the stimulus.

The squat lobster, Galathea strigosa, commonly escapes from threats by swimming with a series of powerful abdominal flexions. Unlike crayfish, however, the abdomen is normally held flexed beneath the cephalothorax in the stationary animal. Thus, in contrast to the crayfish, Galathea escapes with an initial extension, not flexion. Semi-thin sections of Galathea nerve cord show that there are no fibres homologous to the crayfish lateral and medial giants. However, in number, size and position of somata in abdominal ganglia, cobalt backfills of the flexor and extensor motorneuron pools show remarkable similarities to the crayfish system. One fast flexor, which is homologous to the crayfish motor giant in position and size has been studied in more detail. Its morphology and physiology are typical of fast flexor motorneurons and unspecialized compared with the crayfish motor giant.

A preparation has been developed in which recordings can be made from an intact but restrained animal during backward swimming. Extracellular electrodes are used to monitor 2nd and 3rd root activity (nerves to extensor and flexor muscles, respectively). Swimming behaviour can be evoked from a partially deafferented abdominal nervous system either by tactile stimulation of the thoracic carapace or by high frequency electrical stimulation (0.5msec pulses at 100Hz for 250msec) of a 2nd root. Intracellular recordings via Lucifer Yellow filled microelectrodes have been made from neurons active during the post stimulus escape response. This technique allows an investigation of the activity of single neurons involved in escape swimming during the execution of the behaviour itself. Subsequent staining of the cells confirm their identity and allow physiological and anatomical correlations to be made. It is hoped that further experiments of this nature will elucidate the neural mechanisms operative during escape and provide an insight into the generation of patterned motor output by the swimming oscillator. Investigations of this type are hindered in crayfish by the presence of the specialized giant fibre systems for escape.

213.2 INDUCTION AND MODIFICATION OF BURSTING PROPERTIES UNDERLY MODULATIONS OF THE PYLORIC RHYTHM IN ROCK LOBSTERS. Patsy S. Dickinson*and Frédéric Nagy* (SPON: D. J. Prior). Physiology Group, Sch. of Biol. Sci., Univ. of Kentucky, Lexington, KY 40506 and Laboratoire de Neurobiologie Comparée, Place du Docteur B. Peyneau, 33120 Arcachon, France.

Activity in a single interneuron, the anterior pyloric modulator (APM), modifies the output of the pyloric pattern generator, which is located in the stomatogastric ganglion of the rock lobster. Amongst the modulations produced by APM are changes in the amplitude of oscillations of the motor neurons which comprise the pattern generator and changes in the efficacy of certain synapses within the pyloric network (Nagy, F., et al, Neurosci. Letters 23: 167-173, 1981). These modifications appear to result from APM's effects on the ability of the pyloric neurons to generate plateau potentials. We studied these effects on two types of constrictor motor neurons, the LP and PY neurons. The 'burstiness' of the non-pacemaker neurons, such as LP and PY, in the pyloric network depends largely on these regenerative properties (Russell, D. F. and D. K. Hartline, Science 200: 453-456, 1978). APM affects these properties in three ways. Firstly, APM induces the ability to generate plateau potentials in quiescent neurons; secondly, it amplifies these properties in neurons which already exhibit them. In the absence of other factors, this tends to increase the amplitude of oscillations of the neurons involved. Thirdly, APM specifically modifies the repolarizing phase of the plateau potential. This modification is manifested as a reduction in the rate of the active repolarizing phase provoked by an hyperpolarization of the neuron during a plateau potential. This retardation is inversely proportional to the intensity of the current used to hyperpolarize the neuron and provoke the repolarization. Hence the response to a weak hyperpolarizing input during a plateau is slowed down more than that to a strong input. This, together with the induction and amplification of the plateau properties, effectively augments differences in the intensity of synaptic inputs. A strong inhibitory synaptic input will cause a rapid and extensive repolarization of the post-synaptic neuron. The response to a weak inhibitory input will, however, be retarded; if the input is not sufficiently long, a weak input may be unable to bring about full repolarization. Thus the responses to strong inhibitory inputs will be increased, whereas the responses to weaker inhibitory inputs will be decreased. The resulting differential synaptic sensitivity explains the patterns of changes in amplitude of oscillations and in synaptic efficacy seen when APM fires.

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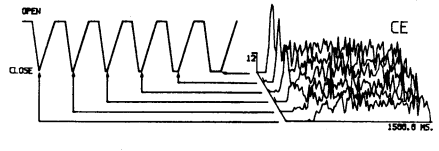
213.3 SUPPRESSION OF CRAYFISH CLAW PROPRIOCEPTOR - MOTOR NEURON INTERACTIONS BY PROPRIOCEPTORS. B. G. Lindsey. Dept. Physiol., Univ. South Florida Med. Ctr., Tampa, FL 33612

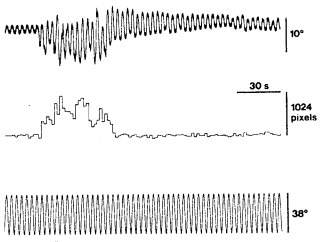
Previous work on crayfish (Procambarus clarkii) claw proprioceptive reflexes has shown that an ensemble of chordotonal organ receptors plays a major role in the generation of both complex proprioceptive fields and coordinated activities of two mutually excitatory claw motor neurons, the slow closer exciter (CE) and opener inhibitor (OI). Intracellular recordings of hyperpolarizing synaptic potentials in CE and OI, revealed by triggering on spikes in closing sensitive proprioceptors (CPs), have suggested that CPs have an inhibitory influence on CE and OI. This work was undertaken to elucidate further variables which might affect CP activity and to determine whether CP activity can alter the excitatory influence of other proprioceptors. Receptors and motor neurons were monitored simultaneously during imposed claw displacements. During claw closing, CP activity increased, while dynamic-static opening sensitive receptor activity decreased. Both changes became more pronounced with increasing displacement velocity and at smaller joint angles. Motor neuron activity evoked by claw opening varied inversely as a function of preceding closing velocity and directly with preceding pause duration at the closed position. Figure shows example of latter observation. Dactyl was alternately closed and opened at constant velocities. Response plane (stack of PST histograms) shows CE activity during imposed claw opening following no pause or pauses of 5 different durations (max. pause: 3 sec.) at the closed position. Plane reveals: 1) that as pause duration increased, time to onset of CE activity decreased, and 2) that an early phasic response appeared and then became more pronounced. Onset time for OI activity varied little (not shown); its early response, present following no pause, tended to increase as pause duration increased. Direct observation of opening sensitive proprioceptors demonstrated that dependence on closing history cannot be explained by changes in opening sensitive receptor activity. These data demonstrate suppression of proprioceptor-motor neuron interactions by CP activity. During claw closing following opening perturbations, concurrent recruitment of CPs and derecruitment of excitatory dynamic-static receptors may serve as a velocity sensitive "dual braking" system to stablilize claw posture. Supported by NS 14934.



213.4 BEHAVIORAL AROUSAL IN THE CRAYFISH PRODUCES A RAPID INCREASE IN OPTOKINETIC GAIN. Richard F. Olivo and Margaret C. Thompson*. Dept. Biological Sciences, Smith College, Northampton MA 01063.

Experiments to measure eye movements in response to visual and proprioceptive stimuli are typically conducted using restrained, passive animals. In the crayfish Procambarus, the passive optomotor response does not stabilize the eyes fully; for sinusoidally oscillating visual and proprioceptive stimuli, the amplitude gain (amplitude of eye movement divided by stimulus movement) is less than 1 and the optokinetic (visually driven) gain alone is less than 0.5 (Olivo & Jazak, Vision Res. 20: 349-53, 1980). During episodes of spontaneous behavioral arousal, however, the gain increases markedly. In the present experiments, we measured optokinetic responses during arousal.

Crayfish were suspended by a post glued to the carapace. The legs and claws were supported by a floating foam rubber ball on which the animals "walked" during periods of arousal. A striped drum around the animal oscillated sinusoidally (38°, 0.05 to 0.34 Hz), eliciting continuous horizontal optokinetic responses that we recorded with a capacitative transducer and a wire wand on the left eye. We monitored leg and claw movements with an overhead video camera connected to a column-scan video digitizer and microcomputer. The computer counted the number of picture elements (pixels) that changed brightness from one video scan to the next (a measure of movement), and output the count as an analog voltage that we recorded on FM tape along with the eye and stimulus movements.



The figure shows a typical episode of arousal. The eye's response (top trace) to a steady 0.3 Hz stimulus (bottom trace) initially has a low quiescent gain (0.07). During the episode of walking (middle trace), the optokinetic response increases by a factor of about 5 (gain 0.33). When walking stops, the response declines slowly and exponentially to a stable level. Analysis of six experiments with distinctly separated periods of arousal shows an increase in gain during arousal by factors of 1.5 to 5, depending in part on the oscillation rate.

Thus, the optomotor system is more effective during spontaneous walking, which presumably is when eye stabilization is required. Furthermore, the optokinetic response to a steady stimulus provides a reliable monitor of the crayfish's level of arousal.

213.5 PROCTOLIN ACTIVATES AND OCTOPAMINE INHIBITS SWIMMERET BEATING. A.G. Bradbury* and B. Mulloney. Zoology Dept., University of California, Davis, CA 95616.

The movements of a crayfish's swimmerets are controlled by a centrally-generated motor pattern that originates in five abdominal ganglia. This motor pattern can be elicited or inhibited by stimulating different command interneurons. We have discovered two pharmacological agents that mimic stimulation of these command interneurons.

We performed these experiments by perfusing isolated abdominal nerve cords of the crayfish, Pacifastacus leniusculus, with buffered saline solutions that contained different test compounds. For bath application, the usual concentration was 10^{-4}M, but for perfusion through the ventral artery we use 10^{-7}M. To monitor the activity of the swimmeret pattern-generators, we recorded impulse traffic in the first roots of each abdominal ganglion; the axons of swimmeret motor neurons run in these roots. We tested each pharmacological agent both with preparations that were silent at the outset and with preparations that spontaneously generated normal motor patterns.

Reagents	No. Expts.	Effect
Proctolin	4	+
Pilocarpine	3	+ & ???
Octopamine	4	-
Acetyl-β-methylcholine	4	???
Dopamine	3	???
l-DOPA	4	???
Serotonin	3	0
Acetylcholine	3	0
n-methyl-dl-aspartate	3	0
dl-aspartate	3	0
GABA	3	0
Glutamate	3	0
Histamine	3	0

+ means activates normal motor pattern
- means inhibits normal motor pattern
0 means no effect
? means alteres spontaneous activity, but not in a behaviorally relevant way.

We will discuss in detail the effects of proctolin and octopamine on the activity of the swimmeret pattern generators.

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213.6 ACTIVATION OF A LONG-LASTING MOTOR PROGRAM BY BAG CELL NEURONS IN APLYSIA. P.H. Brownell* and M.E. Schaefer*. (Spon: S. Moffett) Dept. of Zoology, Oregon State University, Corvallis, Oregon 97331)

In isolated preparations of the abdominal ganglion a burst of impulse activity in the bag cell neurons (the afterdischarge) produces stereotyped and long-lasting changes in the spontaneous activities of identified ganglionic neurons (Mayeri, et al., JNP 42:1165). These neuronal actions are of several types (excitatory and inhibitory), they last several minutes to several hours and they are mediated, in part, by peptides synthesized in the bag cells (Rothman, et al., Nsci Abst 7:636). Bag cells are active prior to the onset of egg-laying behavior (Pinsker, H. and Dudek, F.E. Sci, 197:490). Since many of the neurons influenced by bag cell activity are cells innervating pericardial, branchial and reproductive organs, we are investigating the role of the bag cell system in regulating these organ systems during egg-laying.

To begin these studies we used semi-intact preparations to observe the effect of bag cell activity on spontaneous behavior of the gill and siphon while recording intracellularly from gill and siphon motoneurons (L_7, $LD_{G1,2}$, $L_{9G1,2}$, $LD_{S1,2,3}$). Gill and siphon movements were monitored by a displacement transducer (LVDT) or a photocell and the preparation was perfused with blood or artificial seawater (pH 7.7) at 20°C. During baseline periods (1 hour preceding bag cell stimulation) the gill and siphon were inactive except for synchronous, periodic (1-3/10 min) contractions associated with spontaneous firing of Interneuron II (Int II). When electrical stimulation (1 sec train of electrical pulses applied directly to the bag cell clusters) elicited a bag cell afterdischarge, the frequency and amplitude of Int II-driven contractions of the gill and siphon began to increase within 5 min and remained elevated above baseline levels for 30 min to 1 hr. The increase in frequency of contractions was attributable to an increase in Int II burst frequency. The increase in amplitude of contractions was correlated with prolonged depolarization of gill and siphon motoneurons. These changes were not observed when electrical stimulation failed to elicit a bag cell afterdischarge.

Our results indicate that activation of the bag cell neuroendocrine system causes a long-lasting and stereotyped change in motor activities of the gill and siphon. This motor program appears to be the result of direct actions of the bag cells at two levels (interneurons and motoneurons) within the neuronal circuitry controlling these organs. In the intact animal this motor output should be expressed as an increase in the frequency and amplitude of respiratory pumping during egg laying.

213.7 ANALYSIS OF SWIMMING IN APLYSIA BRASILIANA. David Parsons* and Harold Pinsker. Marine Biomedical Institute, Depts. of Physiology & Psychiatry, UTMB, Galveston, TX 77550.

Aplysia brasiliana swim by alternately opening and closing their bilateral parapodia. Swimming can be elicited by stroking the foot; animals swim continously when restrained above the substrate with hooks through the lateral margins of the foot. Prior to swimming, the foot contracts laterally along most of its length and the parapodia swell. Ligation of the parapodial arteries has no effect on the characteristics of swimming, indicating that the parapodial swelling is due to a redistribution of hemolymph caused by the foot contraction.

Video analysis of free swimming animals (in mid-water) shows a smooth progression throughout the swimming cycle, suggesting that thrust is generated continuously during the parapodial oscillations, and that jet propulsion is not important in thrust generation. Swimming thrust appears to be generated predominately by the strokes of the anterior portion of the parapodia, similar to the way that wing strokes generate lift and forward motion in birds: the configuration of the leading edge and the angle of attack of the parapodia alter consistently such that force vectors can be produced continuously in both upward and forward directions.

5HT (serotonin) can elicit swimming in freely behaving quiescent animals. Eight animals (255-420 gm) were injected (single blind) with either seawater or increasing doses of 5HT, released into the aquarium and observed for parapodial swimming motions. Only 1 animal that received the seawater injections 'swam'; all 8 injected with 5HT (10^{-9} to 10^{-6}M) 'swam.'

Animals do not swim when both cerebro-pedal connectives are cut unless the pedal stump is stimulated electrically, suggesting that the swim pattern generator resides in the pedal ganglion and that the "command" for swimming arises in the cerebral ganglion (von der Porten et al, 1980). If 5HT is injected into the hemolymph of a connective-lesioned animal (circulating concentration approx. 10^{-6}M), it swims continuously for more than an hour before ceasing. Subsequent injections of 5HT elicit similar swim episodes. These findings suggest that 5HT is involved in the initiation and maintenance of the swimming program.

The parapodial musculature consists of several layers of differently oriented muscle fibers concentrated in the anterior third of the parapodia. During restrained swimming both neural activity in small anterior dorsal parapodial branches of nerve P4, and EMG activity in anterior dorsal and ventral muscle fibers is phase locked with the parapodial closing and opening respectively. We are presently recording from smaller nerve branches to allow identification of swimming motoneurons by correlating EMGs with neural activity using digital spike train analysis techniques.

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213.8 THE DISTRIBUTED NATURE OF THE LOCUST FLIGHT MOTOR. R.M. Robertson and K.G. Pearson. Department of Physiology, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada.

It has long been known that locust flight is centrally programmed by the thoracic nervous system and that the motor pattern is organized at the interneuronal level. However knowledge of the structure and properties of flight interneurons is almost totally lacking; what little there is being inferred from motoneuronal recordings. Recently we developed a preparation of Locusta migratoria with which it is possible to record intracellularly from motoneurons and interneurons during the expression of the flight rhythm. Interneurons could be identified on the bases of their physiology and their morphology as revealed by the injection of Lucifer Yellow. To date we have found more than 50 interneurons which are phasically active with the flight rhythm. Some of these have short constant latency connections, both excitatory and inhibitory, with flight motoneurons. In addition, the passage of pulses of depolarizing current into other interneurons can reset the phase of the flight rhythm suggesting that these interneurons are members of the central pattern generator for flight.

The segmental homologies between the wings, muscles and motoneurons of the mesothoracic and metathoracic segments suggest the existence of homologies in interneuronal organization in the two segments. We have identified serially homologous interneurons driving depressor motoneurons of the fore and hind wings and also homologous local interneurons located in the mesothoracic and metathoracic ganglia. However, repetition of interneurons in the mesothoracic and metathoracic ganglia is not a regular organizational feature of this system. Rather, interneurons of the mesothoracic ganglion which can reset the flight rhythm appear to be individuals with no known counterpart in the metathoracic ganglion. Similarly, interneurons in the metathoracic ganglion which can reset the flight rhythm have no mesothoracic homologues, although in this case they can be members of a set of serial homologues with representatives in the first three abdominal ganglia. These observations lead to the conclusion that the flight motor is a system which is distributed within at least six segmental ganglia and operating as a functional unit. This distributed organization of the flight motor may reflect the evolution of flight from a more segmentally distributed behaviour.

This work was supported by the Canadian MRC and by an AHFMR fellowship awarded to RMR.

213.9 MORPHOLOGICAL IDENTIFICATION OF THE 5 MOTOR NEURONS INNERVATING THE DORSAL LONGITUDINAL FLIGHT MUSCLE OF DROSOPHILA. Kazuo Ikeda and J.H. Koenig, City of Hope Research Institute, Duarte, CA. 91010.

The dorsal longitudinal flight muscle (DLM) of Drosophila is a popular preparation for the study of a motor output pattern, since it is composed of only 6 singly innervated fibers. Fibers 1-4 (ventral to dorsal) are each innervated by a different motor neuron, while 5 and 6 are jointly innervated by a 5th motor neuron. These 5 motor neurons were individually backfilled with horseradish peroxidase and positively identified.

Motor neurons 1-4 lie in a cluster located ipsilaterally near the lateral surface of the thoracic ganglion at the border of the pro- and mesothoracic regions, as suggested by Coggeshall, 1978. Since the somata could be backfilled separately, or in combination, it was possible to observe the location of each somata relative to the others in the cluster. Two pairs of closely associated somata were observed in the cluster. The somata of neurons 1 and 2 lie side by side in a horizontal plane with motor neuron 1 in a more anterior position. The somata of neurons 3 and 4 lie slightly ventrally to the somata of neurons 1 and 2, again in a horizontal plane with neuron 3 in the more anterior position.

The soma of motor neuron 5 was located contralaterally in the first layer of the dorsal part of the prothoracic ganglion,close to the midline. (This is not the same cell suggested by Coggeshall, 1978, as being the 5th motor neuron, which is located in the dorso-lateral corner of the ganglion). The shape of the soma, which lay in a horizontal plane, was disc-like, with a diameter of about 8 to 10 μm and a thickness of about 2 μm. A large neurite extends from the soma medio-posteriorly,then makes a sharp turn of almost 90° toward the contralateral side, while sending off extensive dendrites. Finally it enters the posterior dorsal mesothroacic nerve, along with motor neurons 1-4, and innervates muscle fibers 5 and 6. The contralaterality of this neuron is supported by observations on bilateral gynandromorphs (mosaic flies) of the mutant, shibire^{ts1}, which blocks synaptic transmission at 29°C. These flies, which are made up of shi mutant tissue on one side and wild-type normal tissue on the other, show a transmission block of the motor neuron synapses to muscle fibers 1-4 on the shi side, but normal transmssion of motor neuron synapses to muscle fiber 5-6 on that side, and vice versa. Thus, the motor neuron innervating muscle fibers 5-6 must have a contralateral origin, whereas those innervating muscle fibers 1-4 must have an ipsilateral origin. (Supported by USPHS NIH grant NS18856)

213.10 MORPHOLOGY AND PHYSIOLOGY OF MOTOR NEURONES INNERVATING VENTILATORY MUSCLES IN THE ABDOMEN OF THE LOCUST. Q.Z. Yang* and M. Burrows. Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, England.

In the metathoracic ganglion of the locust, nerve 6 carries sensory axons from the tympanum, and motor axons to the median internal ventral muscle and the dorsal longitudinal muscle of the first abdominal segment. Backfilling with cobalt the branch of nerve 6 that innervates the median internal ventral muscle reveals the cell bodies of four motor neurones. Three of these have been identified by simultaneous intracellular recording from the cell bodies and from the muscle fibres, and by subsequent intracellular staining in the central nervous system. Each is an excitatory motor neurone that spikes during the expiratory phase of the ventilatory cycle. Intracellular recording from the muscle has characterized a fourth motor neurone as an inhibitor that evokes IPSPs during the inspiratory phase of ventilation. Two of the excitatory motor neurones have a similar morphology; their cell bodies are ipsilateral to their axons and they have distinct dendritic fields in both the left and right halves of the ganglion. The third excitatory motor neurone has a more anteriorly and medially located cell body. Its dendritic branches are mainly on the side of the ganglion ipsilateral to its axon, though a few cross the middline. Backfilling with cobalt the branch of nerve 6 that innervates the dorsal longitudinal muscle reveals a group of cell bodies on the contralateral side of the ganglion and a further group close to the midline. Neurones of either group may have distinct regions of dendritic branches in both halves of the ganglion.

*On leave from Shanghai Institute of Physiology, 320 Yo-Yang Road, Shanghai, China.

213.11 MOTOR RESPONSE OF THE COCKROACH MESOTHORACIC GANGLION TO GIANT INTERNEURON STIMULATION. M. L. Tobias and R. E. Ritzmann. Dept. of Biol., Case Western Reserve Univ., Cleveland, OH 44106.

Research on the neural circuitry underlying escape behavior in the American cockroach has thus far been restricted to examining outputs from the metathoracic ganglion (T3). It has been demonstrated that giant interneurons (GIs) which receive sensory information via wind receptive hairs on the cerci, subsequently excite motor neurons in T3. The motor output which results from stimulation of a given GI is consistent with leg movements necessary for turning the animal away from a source of wind.

We predicted that the mesothoracic legs would move opposite to the metathoracic legs in order to effectively produce a turn of the animal. Thus, the expectation was that the GIs which excited motor neurons (e.g. levators) in T3 would simultaneoulsy excite the antagonistic motor neurons (e.g. depressors) in the mesothoracic ganglion (T2).

Films of cockroaches turning in response to wind puffs were examined one frame at a time. Statistical analysis indicates a high probability that in any given turn, the mesothoracic leg will move in a direction opposite to that of the metathoracic leg. These findings support the stated prediction.

Electrophysiological experiments consisted of stimulating GIs intracellularly with trains of current pulses, while recording motor neuron activity in the motor roots of the mesothoracic leg. As in similar experiments on the metathoracic leg of the ventral GIs only GI-1 showed a motor output. In both T2 and T3, GI-1 stimulation resulted in weak excitation of the slow depressor motor neuron and the common inhibitor.

Stimulation of the 6 dorsal GIs resulted in unexpected findings. In T3, the output caused by stimulation of a certain GI was invariable (Ritzmann & Camhi, J. comp. Physiol., 125:305, 1978). For example, GI-5 excited the slow depressor motor neuron in every specimen tested. In contrast in T2, stimulation of dorsal GIs can vary from preparation to preparation. For example, GI-5 in T2 can excite depressors, levators or both sets of motor neurons, although the output is consistent within any single preparation.

Experiments currently in progress are designed to explain the variability of motor activity in the mesothoracic ganglion in response to GI stimulation.

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213.12 MUSCLE CELLS IN THE LATERAL HEART TUBES OF THE LEECH: STRUCTURE AND INNERVATION. A.R. Maranto and R.L. Calabrese. The Biological Laboratories, Harvard University, Cambridge, MA 02138.

In the leech, Hirudo medicinalis, a pair of contractile vessels on either side of the animal serve as the hearts. We are engaged in anatomical studies of these vessels to determine how the structure of the muscle cells, their architectural relationships, and their innervation influence the operation of the hearts.

The wall of these vessels consists of an outer layer of thick circular muscle fibers and an inner layer of thinner longitudinal muscle fibers. We have shown with intracellular injection of dyes that the circular and longitudinal fibers are merely different regions of a single type of muscle cell. Every muscle cell contains one circular region which constitutes the external circumference of the vessel and two longitudinal branches which run along the endothelial border of the lumen. The muscle cells may be of either cis or trans form depending on whether their longitudinal branches project in the same or opposite directions. Within each segment there is a specific pattern of cis and trans cell distribution. Additionally, each segment contains a valve region of modified muscle cells with short longitudinal branches.

The muscle cells are dye coupled and freeze-fracture electron microscopy demonstrates the presence of gap junctions between the cells.

The rhythmic beating of the paired hearts is programmed by a well-characterized central pattern generator. When the heart motor neurons are injected with horseradish peroxidase the dye diffuses peripherally into the axon terminals. These are seen to synapse directly onto the muscle cells by electron microscopy. Additionally, a recently discovered heart accessory neuron also extends axons out to the hearts.

We are currently studying the electrophysiology of the muscle cells in situ and in enzyme isolated preparations.

Supported by NSF grant BNS-8108837.

214.1 RELATIONSHIP BETWEEN HIPPOCAMPAL CELL FIRING AND EEG IN THE BEHAVING RAT. G. Buzsáki, C. H. Vanderwolf and L. S. Leung. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, N6A 5C2.

Rats implanted with recording (CA1 and dentate gyrus) and stimulating electrodes, were trained to run in an activity wheel for a water reward. Neuronal activity was recorded by a movable tungsten electrode. Cells were identified by electrophysiological characteristics. All complex spike cells and granule cells fired on the negative portion of the local theta activity. Interneurons in the CA1 region discharged on the positive phase of the CA1 theta waves. About half of the dentate and CA3 interneurons fired on the positive phase of the local theta, the remaining half on the negative phase. In addition to theta dentate interneurons were phase-locked to the 30-50 Hz pattern. Fourier analysis of the interneuronal spike trains showed high power at the theta frequency, coherent with the concurrent EEG. The phase relationship between unit and theta EEG remained unchanged following urethane anesthesia in 11 out of 12 cases (4 dentate, 3 CA1, 1 CA3 interneurons and 3 granule cells). During drinking and immobility monophasic sharp waves of about 100 ms duration appeared in the EEG. These waves were positive in CA1 and negative in the dentate gyrus with a reversal occurring just below the pyramidal layer. Groups of complex spike cells and interneurons fired in bursts during the sharp waves and remained silent between them. During the burst the firing rate of the interneurons increased up to 800 Hz. Constant intensity single volleys in the commissural and perforant path inputs evoked larger number of discharges of the interneurons during running than during drinking or immobility. We suggest that septal pacemaker cells directly excite hippocampal interneurons, which in turn rhythmically inhibit the principal cells generating theta waves. (Supported by NSERC Grant #A0118)

214.2 COMPARISON OF FICTIVE BREATHING AND FICTIVE SWIMMING IN THE LAMPREY. Carl Rovainen, Dept. of Physiology & Biophysics, Washington Univ. St. Louis, MO. 63110

Two of the basic rhythmic behaviors of vertebrates, breathing and swimming, can be generated in the isolated CNS of the lamprey. The problem is to determine the mechanisms of the central pattern generators. The two activities will be compared for common and special features on the basis of published and unpublished observations.

Minimum preparations for pattern generation: Fictive breathing is generated spontaneously by the isolated medulla of the lamprey. Fictive swimming is produced by the isolated spinal cord bathed in 0.2-0.5 mM D-glutamate.

Motor patterns are different: Respiratory motoneurons (MN) in the IX and X nuclei fire synchronously on both sides during 5% of the cycle at 0.3-3Hz. Spinal MN are active during 40% of the swim cycle with strict L-R alternation, rostrocaudal phase lag, and frequency 0.3-2Hz (Exp.Br.Res. 41: 11).

Are MN part of the pattern generator? Intracellular and antidromic stimulation of MN and curare do not change motor patterns in either system. TTX blocks all phasic potential changes in both types of MN. Therefore, MN do not appear to produce the basic rhythms.

Synaptic potentials in MN: Short bursts of EPSPs drive respiratory MN, but Cl^- injections show few IPSPs between them. In contrast, spinal MN have sinusoidal changes in membrane potential due to modulated EPSPs and Cl-sensitive IPSPs (Acta Physiol.Scand. 108: 9A).

Effects of blocking synaptic inhibition: Synchronous bursts similar to those during breathing and distinct from longer seizures are still generated by the brain bathed in 30 µM picrotoxin plus 20 µM strychnine or in Cl-free fluid. In contrast, both low-Cl fluid and 5 µM strychnine disrupt fictive swimming, and 1-2 µM strychnine increases frequency of bursts (Acta Physiol.Scand. 110: 103).

Complete midline sections reduce respiratory MN activity, but increase spinal MN activity and disrupt fictive swimming.

Proposed models for pattern generators: The basic rhythms of both fictive breathing and swimming appear to be generated by interneurons (IN), while MN relay the patterned activities to muscles. The generator IN for both rhythms are likely to be duplicated on both sides of the CNS. The respiratory generator IN are probably coupled by excitatory synapses and may have intrinsic bursting properties. The generator IN for swimming are probably coordinated by reciprocal inhibition across the midline. Possible generator mechanisms will be discussed.

214.3 COMPARISON OF HATCHING AND WALKING MOTOR OUTPUT PATTERNS IN NORMAL AND DEAFFERENTED CHICKS. A. Bekoff. EPO Biology Dept., Univ. Colorado, Boulder, CO 80309.

This study was designed to explore the possibility that the neural pattern generating circuitry that is used to produce the leg movements of hatching in the chick embryo is re-used later to produce walking in the posthatching chick. Previous work has shown that the pattern generating circuitry for the leg movements of hatching is present and functional long after hatching has been completed and at a time when the chick is using walking as its normal mode of locomotion (Bekoff & Kauer, Neurosci. Abstr. 6:75, 1980). Thus we can eliminate the possibility that the circuitry for hatching leg movements becomes permanently modified to produce the walking circuitry. To begin to explore the possibility that one pattern generator could be transiently modulated by sensory input to produce the two different motor output patterns, the following experiments were carried out.

First, the leg motor output patterns typical of hatching and walking were each characterized using electromyogram (EMG) recordings. Quantitative analysis of the EMG recordings from muscles within a limb (intralimb coordination) revealed that there were basic similarities between the two behaviors in that, in general, muscle pairs showed the same pattern of coordination in both (e.g., alternation or coactivation). However, there were also significant differences in parameters such as mean period length, burst durations, phase relationships and in the relationships among these parameters.

It is reasonable to assume that different patterns of input from leg sensory receptors are present during hatching and walking. If these are responsible for modulating the output of one pattern generating circuit, then removing all sensory input from the legs ought to result in a convergence of the motor output patterns seen during hatching and walking. To test this, deafferentation of the legs was carried out in posthatching chicks by cutting the lumbosacral dorsal roots bilaterally.

These deafferented chicks were readily induced to walk on a treadmill and to hatch in a glass egg. While some differences between the motor output patterns remained, many of the differences decreased dramatically. In general, the walking motor output became more similar to hatching rather than the reverse. These data thus support the hypothesis that sensory input from the legs may normally modulate the output of one pattern generating circuit in order to produce the two different motor output patterns that are typical of intralimb coordination during hatching and walking.

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214.4 DO SERIAL DEPENDENCIES IN NEURONAL SPIKE-TRAIN INTERVALS PRELUDE RANDOMNESS? J. J. Sherry and W. R. Klemm. Depts. Biol. and Vet. Anat., Texas A & M Univ., College Station, TX 77843.

We have demonstrated that spike-train intervals have Markovian dependencies (Brain Res. Bull. 8:163, 1982), i.e. the value of a given interval affects the value of up to 5 succeeding intervals. Since interval distribution is not independent, it is important to establish whether or not intervals can still occur randomly (given a probability density function (PDF), the chance of any interval occurring is equal (i.e. occurrence depends only on the PDF)). While it is generally assumed that spike-train intervals are random, this hypothesis has not been formally tested. One way to test this hypothesis is to utilize Chi-square methodology to compare two or more PDFs using the quantile model (Parzen, 1979).

Randomness can be evaluated by dividing the train of intervals into segments by some unvarying rule. For example, one might construct a series of histograms such that each histogram contains every fifth interval (i.e. intervals 1, 6, 11, etc. in the first histogram, 2, 7, 12 in the second, etc.). These histograms are converted into cumulative PDFs by summing the counts in each bin in a histogram and then dividing the cumulative total in each bin by the total number of intervals in that segment. Appropriate quantiles are chosen (i.e. decatiles; 10%, 20%, etc.) and the msec value of each decatile in the first segment is noted. The second and succeeding cumulative PDFs are examined to identify the percentiles that are associated with the msec values of the first histogram. Chi-square testing is based on observed values which are the differences between each succeeding percentile in the second histogram and the expected values of 10% are taken from the first histogram (d.f. = number of quantiles - 1 or in this case, 9 - 1 = 8).

Interspike intervals recorded from 8 cerebellar neurons were found to be random sequences when evaluated by this method (none of the PDFs were significantly different). However, the intervals from these neurons diverged markedly from statistical independence and also demonstrated Markovian dependencies. Clearly, randomness and independence may reflect different phenomena and must be evaluated separately.

This Chi-square quantile model can also be used to evaluate stationarity, where one would divide a train of interspike intervals into equal segments (halves, thirds, or fourths, for example). Each segment can be used to generate cumulative PDFs which can be compared by Chi-square evaluation of quantiles. The spike-trains used for the evaluation of randomness were evaluated for stationarity. Their Chi-square values ranged from 1.61 to 5.04, which were non-significant, which means that these segments were stationary.

214.5 POST-INHIBITORY REBOUND IN GASTROPOD NEURONS. R. D. Longley* and A. J. Longley. Pacific Sciences Institute and Friday Harbor Laboratories, Friday Harbor, WA 98250.

Post-inhibitory rebound is a ubiquitous phenomenon in both vertebrate and invertebrate neurons. When coupled with inhibitory input it contributes to oscillatory activity. It is a prominent feature of the feeding rhythm in the buccal ganglion neurons of gastropod mollusks where certain neurons produce a burst of spikes on rebound from an inhibitory input.

Using voltage-clamp techniques on neurons with axons attached, we have examined this post-inhibitory rebound (PIR) current in bursting neurons and buccal ganglion motoneurons in the nudibranch Tritonia diomedia. On return to resting potential from a negative voltage step, a PIR current could be demonstrated in these neurons, but in some cases this current competed with a transient potassium outward current. In neurons where this outward current was present, the PIR current was masked, except at intermediate levels of the hyperpolarizing conditioning step which did not remove the inactivation of the transient potassium current. In buccal ganglion motoneurons, this transient potassium current may be completely absent.

The PIR current was similar in the buccal ganglion motoneurons B1 and B5, but in B5 it was much more effective in producing a burst of spikes. This seems to be due in part to a different neuron shape and membrane resistance. In these neurons an anomalous rectification current was found with a time constant of 1-2 sec for steps to voltages more negative than -65 mv. This time dependent anomalous rectification was superimposed on a PIR current with a similar time constant. The amplitude of the PIR current, which persisted on return to resting potential, varied linearly with the conditioning voltage step and reversed sign at resting potential to become an outward current for positive steps. In the excised ganglion, B5 received spontaneous inhibitory input which hyperpolarized the soma 15-20 mv for about 30 sec. A current of 20-30 na was required to hold the soma clamped at resting potential during this time. Following this inhibitory input, with the soma held at resting potential, a PIR current of 1-2 na was generated in the axon, which was comparable in time course and amplitude to that obtained with a -20 mv step from resting potential in the soma.

These results suggest that the PIR current may be generated in the axon and is most effective in neurons where the transient potassium current is absent. The ability of the PIR current to produce a burst of spikes may also depend on neuron resting potential and resistance, as it does in B1 and B5.

214.6 ARTIFICIALLY CROSS-COUPLED TONIC NEURONS PRODUCE ALTERNATING BURSTS. D.K. Hartline and D.V. Gassie* Bekesy Laboratory, University of Hawaii, Honolulu, HI.

Theoretical studies predict that reciprocal inhibition between two tonically firing neurons can produce alternating bursts of spikes if synaptic and decoupling (e.g. adaptation) parameters are within appropriate ranges. An approximate condition for bursting is $1+\xi/\rho<\sigma<1/\rho$ (ξ= ratio of synapse to adaptation decay time-constant; ρ= ratio of initial to final firing frequency for adaptation; σ= strength of IPSP: Hartline, Roberts and Baker, Neurosci. Abstr. 6:406). Typical values for crayfish stretch receptor (MRO) and its inhibitor fiber were $\xi<0.01$ and $\rho\doteq0.8$, indicating a critical range for σ between 1.0 and 1.2. Appropriate values of σ were obtained with single IPSPs at late phase in a postsynaptic train, or when two IPSPs were summed. Artificial reciprocal inhibition between two MROs was produced by cross-coupling the spikes of each to the inhibitory fiber of the other through stimulators. With weak IPSPs, no bursting occurred; the two spike trains tended to lock in antiphase. With stronger inhibition, alternating bursts resulted, as predicted (Fig. 1). Various patterns were observed (e.g. Fig. 2). Two rhythms were often seen, a short-period (1-10 sec) and a long-period (several minutes) one, the latter evidently due to long time-constant processes. Substantial variability was found in burst patterns. Normal networks can possess intrinsic burst-generating properties and/or redundant mechanisms that may provide stability. This system may therefore offer insights into properties of reciprocally-coupled neurons removed from network complexities.

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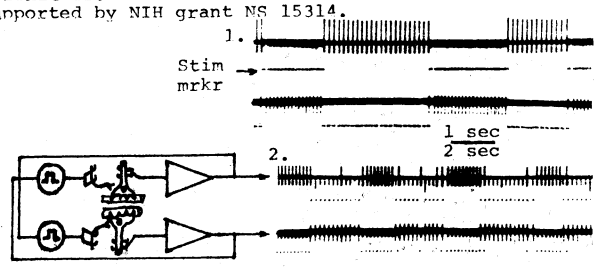

214.7 CIRCUITRY OF THE LEECH VISUAL SYSTEM. E.L. Peterson. The Biological Laboratories, Harvard Univ., Cambridge MA (present address: Dept. of Biology, McGill Univ., 1205 Ave. Dr. Penfield, Montreal H3A 1B1).

The medicinal leech has 5 bilateral pairs of eyes arranged in 2 rows on the dorsal surface of the head (numbered 1-5 beginning with the most anterior pair). The physiology of the eyes themselves is known from earlier work (reviewed, Kretz, et al., J. comp. Physiol. 106, 1 (1976)). Each eye contains 30-50 photoreceptor cells, each of which sends an axon into the associated optic nerve. (It is assumed, though not yet shown properly, that photoreceptor axons extend through the optic nerves and into the CNS.) Here I report a preliminary analysis of the neural circuitry processing visual input to the CNS. I have identified about 20 pairs of neurons in the CNS which respond at fixed, short latency to illumination of the eyes. One pair, the LV (lateral vision) neurons, has been examined in detail. An LV neuron has its soma in the first segmental ganglion, and has a contralateral axon which projects rostrally to the midline of the supraesophageal ganglion. A light pulse produces a sharp depolarizing transient 'on' response, followed by a plateau, which is sustained by a continuous barrage of PSPs that lasts throughout the pulse. This response parallels that of the photoreceptor cells almost exactly. Only the ipsilateral eyes 3-5 excite an LV neuron. The excitatory response persists in 40 mM Mg^{++} saline a solution which permits only electrical synaptic transmission in the leech CNS. Moreover, when an LV neuron is injected with the fluorescent dye Lucifer yellow additional fibers dye-coupled to the LV neuron are stained. A broad diffuse tract, apparently containing many fine axons, courses forward, bifurcates, and exits through the ipsilateral nerves D3 and D4. Nerves D3 and D4 lead to eyes 3,4 and 5.

When any of the contralateral eyes 3-5 are illuminated the LV neuron is inhibited. This is a clear case of lateral inhibition --a basis of feature detection in most visual systems. The obvious possibility--that lateral inhibition is mediated by reciprocal inhibition between the LV neurons--has been tested and rejected as a mechanism.

Thus the LV neurons appear to be second order sensory neurons. The sidedness of their response suggests that they will turn out to be interesting behaviorally.

215.1 SUSTAINED INCREASE IN GLUTAMATE RELEASE ASSOCIATED WITH LONG-TERM POTENTIATION OF PERFORANT PATH IN VIVO. A.C. Dolphin*, M.L. Errington* and T.V.P. Bliss* (SPON: ENA). Division of Neurophysiology and Neuropharmacology, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

The relative extent to which pre- and post-synaptic mechanisms are involved in the genesis and maintenance of long-term potentiation (LTP) in the hippocampus is an unresolved question. By attaching recording electrodes to a push-pull cannula we have been able to perfuse the synaptic region of the dentate gyrus of the urethane-anaesthetised rat, while recording field potentials evoked by stimulation of the perforant path in the granule cell body region. We have investigated the effect of a single high-frequency train (250 Hz for 500msec), designed to induce LTP in the perforant path, on the release of (^{3}H)-glutamate, synthesised from continuously perfused (^{3}H)-glutamine, and subsequently separated from (^{3}H)-glutamine and (^{3}H)-GABA by ion-exchange chromatography. Induction of LTP, as monitored electrophysiologically by measuring the synaptic component (population epsp) of the evoked response, was accompanied by a prolonged increase (>1 hour) in the release of (^{3}H)-glutamate. Perfusion with Ca^{++}-free medium led to a reversible abolition of the evoked response, as did perfusion with the glutamate antagonist, D-γ-glutamylglycine. These results provide additional evidence for a transmitter role for glutamate in the perforant path and suggest that an important component of LTP is presynaptically mediated.

215.2 NORADRENERGIC MODULATION OF LONG-TERM SYNAPTIC POTENTIATION IN THE HIPPOCAMPUS. William F. Hopkins and Daniel Johnston. Neuroscience Program, Baylor College of Medicine, Houston, TX 77030.

Long-term potentiation (LTP) of synaptically-mediated responses in the hippocampus has attracted considerable attention as a possible cellular substrate for information storage in the central nervous system. For a number of reasons the mossy fiber synapses onto the CA3 subfield may be particularly favorable sites for studies of the biophysical mechanisms underlying LTP (cf. Brown and Johnston, this volume). The CA3 region is densely innervated by noradrenergic fibers primarily from the locus coeruleus. We were interested in whether this rather diffuse projection into the CA3 region might play a modulatory role in the induction or maintenance of LTP of the mossy fiber input.

Rat hippocampal slices were maintained in vitro using standard techniques. Field potentials were recorded from stratum pyramidale in response to stimulation of the mossy fiber pathway. LTP was typically induced with 100 Hz, 2 s trains of 0.05 ms pulses, delivered at 2-3X the test pulse stimulus intensity. the fractional increase in the field response was measured as a function of time following the conditioning train. In all our experiments this fractional increase in the field response decayed exponentially; a time constant of greater than 15 min was taken as evidence of LTP.

When the field response was monitored at 0.2 Hz following the induction of LTP, the mean decay time constant was 36±10 (SEM) min. The addition of 10 uM norepinephrine to the bath increased the decay time constant nearly two-fold to 66±18 min. This prolongation of LTP by norepinephrine was readily reversible. When the field response was monitored at 10 min intervals, rather than at 0.2 Hz, following the conditioning train, the mean decay time constant was larger (87±67 min). Norepinephrine added to the bath (10 uM) also reversibly increased this decay time constant (313± 122 min). In the absense of the conditioning train for LTP, we found that the field response consistently displayed low-frequency depression after several minutes of stimulation at 0.2 Hz. Norepinephrine accentuated this depression when added to the bath at a concentration of 10 uM.

These effects of norepinephrine on the mossy fiber evoked field responses may provide useful information for the mechanisms of use-dependent synaptic plasticity in the hippocampus. (Supported by McKnight Foundation Neuroscience Development Award and NIH Grants NS11535, NS15772 and NS18295.)

215.3 FEED-FORWARD INHIBITION AND THE REGULATION OF CELL DISCHARGE FOLLOWING LONG-TERM POTENTIATION. S. Brassel*, W. B. Levy, and O. Steward. Dept. of Neurosurgery, University of Virginia School of Medicine, Charlottesville, VA 22908. SPON:Nancy L. Desmond

Brief high frequency activation of the entorhinal cortical (EC) projection to the dentate gyrus (DG) results in long-term potentiation (LTP) of synaptic efficacy of EC-DG synapses. Douglas (Neurosci. abs. 4,470,1978) reported that LTP can be prevented if potentiating stimulation of the EC is delivered concurrently with high frequency stimulation of the dentate commissural pathway (Com). In exploring this phenomenon in more detail, we obtained evidence which suggested that the Com stimulation did not block LTP, but rather altered the way that the granule cells discharge in response to EC stimulation.

We recorded the extracellular EPSP evoked by EC activation, and the population spike which reflects the summed discharge of dentate granule cells. Following potentiating stimulation of the EC alone, LTP was reliably observed. When EC stimulation occurred simultaneously with Com stimulation, there was little or no subsequent increase in the population spike but EPSP potentiation was observed. These results suggested that cell discharge following LTP might be modulated independently of the changes in direct excitatory synaptic drive.

To evaluate any changes in the relationship between excitation and cell discharge, we defined the relationship between population EPSP and population spike across a wide range of stimulus intensities before and after potentiation. Previous studies (Wilson, J. Neurophys. 46:324-338,1981) have revealed that LTP induced by EC stimulation alone results in a change in the way that granule cells translate EC excitation into cell discharge; after LTP, an EPSP of a given magnitude generates a larger population spike than an EPSP of an equivalent magnitude prior to LTP. After concurrent high frequency activation of EC and Com, the relationship between EC excitation and cell discharge also changed but in the opposite direction, viz. an EPSP of a given amplitude generated a smaller population spike than prior to potentiating stimulation.

The previous demonstration of a dissociation of the EPSP/spike relationship in the EC-DG system following LTP suggested a feed-forward inhibitory circuit which regulated granule cell discharge to EC activation. We hypothesized that the EC-DG synapse potentiated to a greater extent than the feed-forward inhibition (Wilson, Levy & Steward, J.Neurophys. 46:339-355,1981). The present results suggest that this feed-forward inhibitory action may account for the effects of concurrent Com activation on EC-DG LTP. Concurrent EC and Com stimulation may potentiate the feed-forward inhibition resulting in a smaller population spike for a given EPSP. Supported by NS12333 & RCDA NS00325 to O.S.

215.4 EFFECT OF COLCHICINE ON SYNAPTIC TRANSMISSION AND LONG-TERM POTENTIATION IN THE DENTATE GYRUS. T. Sutula,* R. Goldschmidt,* and O. Steward. Depts. of Neurosurgery, Neurology & Anatomy, Univ. of Virginia, Charlottesville, VA 22908.

Brief trains of stimuli to certain pathways in the hippocampal formation result in long-term potentiation of synaptic efficacy (LTP). Previous studies have revealed changes in the size (Fifkova, van Harreveld, J. Neurocyt. 6:211-230, 1977) or shape (Desmond, Levy, Anat. Rec. 199:68A, 1981) of dendritic spines, and changes in the number of shaft synapses (Lee, Schottles, Lynch, J. Neurophysiol. 44:247-258, 1980) after the induction of LTP. If dendritic shape is altered by LTP, it seems possible that this change would involve the cytoskeleton. Thus, agents which disrupt the cytoskeleton might disrupt LTP. This study examines the effects of colchicine and colcemid on LTP.

LTP was examined in the entorhinal cortex (EC) to dentate gyrus (DG) projection system. Bilateral stimulating and recording electrodes were placed in the EC and DG. Baseline responses were recorded prior to unilateral injection of colchicine into the DG. The contralateral electrode pair served as an intra-animal control.

Injections of 2.5μg of colchicine in 0.5μL of water resulted in dramatic increases in granule cell excitability, as evidenced by increases in population spike amplitude, and the appearance of multiple spikes. These changes persisted for 4-6 hours. Despite the fact that the potentials appeared relatively normal at 6 hours post-injection, LTP was consistently blocked. On the control side, LTP was reliably observed.

It was of interest whether colchicine's effect on LTP reflects selective neurotoxicity for granule cells. Colcemid, which disrupts microtubules but is not toxic to granule cells, resulted in an increase in excitability and LTP was not blocked. We also examined the effects of colchicine on LTP in the hippocampus proper, where colchicine has minimal cytotoxic effects. Colchicine had similar effects in the commissural projection to CA1 in that there was a similar increase in excitability, and LTP at 6 hours was disrupted. Because colchicine also blocks LTP in CA1, where there are few neurotoxic effects, and because the disruption of LTP in the DG occurs before evidence of morphological changes, we feel that the blocking of LTP is not directly related to cell death. We propose that the increase in excitability immediately post-injection reflects disruption of the dendritic cytoskeleton (particularly the collapse of the dendritic spines) and that the inability to induce LTP is a consequence of the disruption of the cytoskeletal machinery which is necessary for the changes involved in LTP.

Supported by NS12333 & RCDA NS00325 to O.S., and a Grass Foundation fellowship to T.S.

215.5 THE EFFECT OF CHRONIC ETHANOL INGESTION ON SYNAPTIC DISTRIBUTION AND POTENTIATION IN RAT DENTATE GYRUS. C.J. Rogers, W.C. Abraham, B.E. Hunter and D.W. Walker. Dept. of Neuroscience, Univ. of Fla. Coll. of Med. and VA Med. Ctr., Gainesville, FL 32610.

This study investigated the persistent effects of chronic ethanol exposure on the rat dentate gyrus by the electrophysiological analysis of synaptic distribution and potentiation of entorhinal cortex (EC) afferents to stratum moleculare (SM) of the dentate gyrus (DG). Rats were maintained on nutritionally-complete ethanol (Group E) or sucrose-containing liquid diets (Group S) for 20 weeks. Rats were withdrawn from the special diets at least 8 weeks prior to acute electrophysiological recordings. Concentric bipolar electrodes were placed in the angular bundle (AB) to co-activate the medial and lateral EC afferents to SM of the DG. Extracellular field potentials in the DG were recorded with micropipettes (3-5 µm tip). Input/output (I/O) curves were generated with the recording electrode at a fixed point 150 µm ventral to the inversion point. Laminar analyses were then obtained by stepping the recording micropipette in 25 µm increments across both blades of the DG, and sampling the field potentials at each point in response to AB stimulation. Current-source density (CSD) profiles were calculated from the field potential data. After field potential data were obtained, the recording electrode was returned to the fixed recording site in the hilus and potentiation processes were examined. Paired-pulse potentiation (PPP), frequency potentiation (FP), and long-term potentiation (LTP) of the EC-DG synpases were examined.

Chronic ethanol treatment (CET) significantly reduced the width (20%) of the current sink in the SM of both blades of the DG. The shrinkage was confined to the distal portion of the sink indicating a loss of EC afferents in the outer SM. This suggests that CET produces a preferential loss of lateral EC synapses onto DG granule cells. CET did not significantly alter short-term potentiation processes (PPP and FP) in the DG or the I/O functions of the basic EPSP and PS measures. However, when the PS was expressed in proportion to the EPSP (PS/EPSP), Group E was found to have a significantly reduced (20%) PS/EPSP ratio. Basic synaptic and PS responses were unchanged during LTP. However, when LTP was expressed as a PS/EPSP ratio, a significant decrement in LTP was observed in group E rats.

The results of these experiments suggest that CET 1) decreases the spatial distribution of lateral EC afferents to DG and 2) decreases the excitability of granule cells in response to equivalent EPSPs and during LTP. This latter change may result from ethanol effects on the "synchrony" of granule cell unit activity or a reduction in the granule cell population.

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215.6 **EFFECTS OF ANTI-S100 SERA ON LONG-TERM POTENTIATION IN THE HIPPOCAMPAL SLICE.** D.Lewis, V.E.Shashoua, T.J.Teyler & B.W.Moore. Program in Neurobiology, NE Ohio Coll. Med., Rootstown, OH 44272, Dept. Biological Chemistry, McLean Hosp./Harvard Med. Sch., Belmont, MA 02178 & Dept. Psychiatry, Washington U. Sch. Med., St.Louis, MO 63110.

The brain specific protein S100 has been implicated in the neural mechanisms of learning and memory. S100 begins to accumulate in the rodent hippocampus on day 7, a time when long-term potentiation (LTP) is first seen in the CA1 region of the hippocampus. Hippocampal LTP is associated with the secretion of protein into the extracellular space and S100 is present in hippocampal extracellular fluid. We sought to test the possibility that S100 is involved in the production of LTP.

Rat hippocampal slices were prepared according to standard procedures. Population responses were recorded from the CA1 region in response to st. radiatum stimulation. In the first set of experiments, after baseline determination, the normal Earles media was equal-volume exchanged for media containing diluted (1:10 to 1:40) anti-S100 whole sera. Tetanic stimulation (33Hz/3sec) which produced a doubling of population spike amplitude in normal media, did not result in LTP in the presence of the anti-S100.

In the second set of experiments a within slice control procedure was employed wherein 2 recording electrodes were placed in the CA1 cell body layer. A stimulating electrode was positioned between the two recording electrodes in st. radiatum. A pressure ejecting micropipette was placed near one recording electrode for delivery of test substances. Anti-S100 whole sera diluted (1:5 to 1:10) with media and fast green was ejected onto one recording site. Subsequently, tetanic stimulation (100Hz/1sec) was delivered to st. radiatum. Both post-tetanic potentiation (PTP) and LTP were assessed at both electrodes for up to 30min post-tetanus. Anti-S100 sera had no effect on PTP but blocked LTP. Control recording in the same slice showed both PTP and LTP.

We report that anti-S100 sera blocks LTP but not PTP. The following control procedures did not block PTP or LTP: vehicle, pre-immune sera & gamma globulin. This provides support for our hypothesis that the brain specific protein S100 is somehow involved in the mechanism of LTP. (Supported by the NSF.)

215.7 POSTNATAL DEVELOPMENT OF TRANSIENT AND LONG-TERM POTENTIATION IN THE NEOCORTEX AND DENTATE GYRUS OF THE RAT. D.A. Wilson* & R.J. Racine (SPON: H. Weingarten). Dept. of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

The postnatal development of short-term potentiation (STP) and long-term potentiation (LTP) were examined in the neocortex and the dentate gyrus of anesthetized rats, aged 7 days (PN7) to adult (PN180).

Biphasic stimulation (0.1 msec each pulse, 50-1000uA intensity) of callosal fibers produced a biphasic, positive-negative, transcallosal response (TCR) recorded near the surface of the anterior neocortex in all age groups. The TCR showed a decrease in threshold, latency, and halfwidth, and an increase in peak amplitude with age. Test pulses producing sub-maximal responses (50% of maximum on the I/O curve) were presented at 0.1 Hz for 10 min preceding and 10 min following a single high intensity train (1200uA, 400 Hz, 100 msec). A train with these parameters reliably produces STP and LTP of the mature TCR (Wilson & Racine, Soc Neurosci Abst, 1981, 7, 69). STP and LTP in young animals, however, could not be reliably detected until after PN16 and PN18, respectively. The degree of STP and LTP was initially small but approached adult levels one to two weeks after their initial appearance.

Stimulation of perforant path fibers produced a positive excitatory post-synaptic potential (epsp), recorded in the dentate hilus, from PN7 to adult. The epsp showed a decrease in threshold, latency and halfwidth, and increase in peak amplitude with age. Population spikes could be produced in all age groups, although they were generally of small amplitude at PN7. STP and LTP could be produced in this system in mature animals, as reported elsewhere (Bliss & Lømo, J. Physiol, 1973, 232, 331-356; Douglas & Goddard, Brain Res, 1975, 86, 205-215). In the immature dentate, STP and LTP could not be reliably detected until the second postnatal week, with STP appearing prior to LTP. Again STP and LTP approached adult levels over the course of one to two weeks after their initial appearance.

The maturation of the evoked response (e.g. threshold and latency) did not correlate closely with STP/LTP development in either the hippocampal or neocortical system. Also, the correlations between STP/LTP development and structural developments such as synaptogenesis, spine formation, or myelinogenesis (as reported in the literature) were not particularly strong in either system. These results suggest that the postnatal development of STP and LTP, and thus the mechanism of potentiation in mature animals, may not depend so much on the maturation of specific structures (e.g. dendritic spines) as on the maturation of neurochemical processes (e.g. Ca++ binding or protein phosphorylation).

215.8 COOPERATION AMONGST AFFERENTS TO THE STRATUM RADIATUM OF CA1 FOR LONG-TERM POTENTIATION OF EVOKED RESPONSES, K.S. Lee
Max Planck Institute for Psychiatry, Munich, West Germany

An enhancement of evoked potentials following brief bursts of afferent activity (long-term potentiation;LTP) has been shown in several systems in the hippocampus and recently in the cerebral cortex. This effect can persist for days or weeks and has been suggested as a possible substrate for associative memory. With respect to the induction of LTP, a cooperation amongst entorhinal cortical afferents to the dentate gyrus has been shown by McNaughton (Brn.Res.157:277,1978), while a negative interaction between commissural and entorhinal afferents was demonstrated by Goddard and Riives (Neurosci.Abs.7:773,1981). The present study sought to examine if afferents to the stratum radiatum (s.r.) of CA1 exhibit similar interactive characteristics. Test stimuli were delivered by an electrode in the s.r. near the CA1-CA3 border (OS/C) and recordings were taken in the s.r. of CA1. Coactivation of afferents was achieved by: 1)placing another stimulation electrode in the subicular aspect of the s.r. or 2)increasing the duration of individual stimulation pulses delivered to the OS/C (during conditioning only). In addition, the effect of coactivation of the postsynaptic pyramidal cells was examined by antidromic activation from a stimulating electrode in the alveus. Conditioning was delivered to either the OS/C stimulator alone or in conjunction with one of the other stimulators. Conditioning trains were as follows: 3 bursts of 50 pulses at 250 sec $^{-1}$, with bursts separated by 5 seconds. Test pulses were delivered at 5 seconds intervals to the OS/C stimulator alone.

When compared to the amount of enhancement obtained with OS/C conditioning alone, coactivation of OS/C and the subicular electrode (AS/C) elicited a significantly greater increase of the OS/C responses at both 5 and 20 minutes post-conditioning. The presentation to OS/C alone of conditioning stimuli with longer individual pulse duration resulted in an enhancement which was greater than that obtained with any of the other stimulation paradigms. In contrast, the amount of enhancement following coactivation of the antidromic (alvear) and OS/C stimulators did not significantly differ from that obtained with OS/C conditioning alone.

It therefore appears that afferents to the s.r. of CA1 cooperate with respect to the amount of LTP induced. The firing of the postsynaptic cell, however, does not appear to be a critical factor in this cooperation since antidromic coactivation did not increase the amount of LTP. The possiblity that temporal relationships other than coactivation result in the modulation of LTP is currently under investigation.

215.9 QUANTITATIVE ELIMINATION OF LONG-TERM POTENTIATION (LTP) IN THE HIPPOCAMPUS BY DISSOCIATIVE ANESTHETICS. J.L. Stringer* and P.G. Guyenet (SPON: C.E. Creutz). Univ. of Virginia, Sch. of Medicine, Dept. of Pharmacology, Charlottesville, VA. 22908.

CA1 population spikes (PS) elicited by stimulating the contralateral CA3 field were recorded in urethane-anesthetized, artificially respirated Spraque-Dawley rats. In each animal the curve relating PS size and stimulus intensity (input-output curve) was established. A stimulus intensity which produced a 2-4 mV PS was selected and a train of 20 stimuli (50Hz, 500msec) was delivered at this current intensity. The high frequency train was followed by a reproducible shift to the left of the linear portion of the input-output curve which was stable for at least one hour and, therefore, represents a form of LTP. Since the potentiated curve was parallel to the initial response the amount of LTP was quantitized by measuring the vertical distance, in mV, between the two lines. This value in the control animals was 6.5 ± 1.0 mV (mean ± S.E.M., n=5).

The intravenous administration of the dissociative anesthetics phencyclidine (PCP) and ketamine resulted in a dose-dependent shift to the right of the input-output curve. Following administration of these drugs, a stimulus intensity which produced a 2-4 mV PS was selected and a train of 20 stimuli (50Hz, 500 msec) was applied in CA3. In PCP pretreated animals the left shift of the input-output curve induced by the high frequency burst was decreased in a dose-dependent manner (ED_{50}=3 mg/kg). At a dose of 6 mg/kg of PCP, and 30 mg/kg of ketamine, LTP was totally abolished.

These experiments raised the question of whether the loss of LTP could be related to the use, during the high frequency train, of a higher stimulus intensity in the drug-treated as compared to the control animals. To test this hypothesis, the same experimental paradigm was used in a series of rats pretreated with two drugs unrelated to the dissociative anesthetics-diazepam and sodium thiopental. These drugs also cause a shift to the right of the input-output curve very similar to that observed with the doses of PCP and ketamine that were used. In these animals a train of 20 stimuli (50Hz) identical to that described above was delivered in CA3. This was followed by a left shift of the input-output curve of identical magnitude as that observed in drug-free animals (shift measured on mV axis, mean ± S.E.M.: 8.3 ± 2.3 mV for up to 4.0 mg/kg diazepam, n=4; 6.4 ± 1.3 mV for up to 40 mg/kg thiopental, n=4). This result demonstrates that the use of a higher intensity during the high frequency train does not account for the abolition of LTP after pretreatment with PCP or ketamine. It is therefore concluded that PCP and ketamine specifically interfere with the mechanism responsible for the generation of LTP. (NIDA #02310)

215.10 LONG-TERM POTENTIATION IN THE DENTATE GYRUS: COOPERATIVITY BETWEEN SEPTAL AND ENTORHINAL AFFERENTS. G. Robinson* and R.J. Racine. Dept. of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

Previous investigators have shown long-term potentiation (LTP) of the perforant path-granule cell (PP-GC) synapse to be a cooperative process requiring coactivation of a critical number of PP fibres (McNaughton, B., Douglas, R., & Goddard, G., Brain Res., 157: 277, 1978). Such a cooperative interaction between neural pathways, in the production of LTP, may serve as a model of associative memory. We have examined cooperative interaction effects between the entorhinal (perforant path-PP) and septal (septodentate-SD) afferents to the dentate gyrus granule cells in the anesthetized rat.

Test pulses (0.1), sufficient to evoke a small population spike, were applied to the PP both before and after the application of high-frequency trains (400 Hz, 50 ms). Four sets of 3 trains were applied separately to the SD and PP fibres before activating both pathways simultaneously. The LTP effects produced by separate train applications were driven to saturation before applying the trains concurrently to both pathways.

Separate activation of SD fibres resulted in a small transient heterosynaptic increase in the amplitude of the PP-GC population spike but had no measurable effect on the population EPSP. This increase, not evident in all animals, had usually decayed to pretrain levels within 3 min. Activation of the PP resulted in a significantly greater potentiation, including LTP, of both the PP-GC population spike and EPSP. This effect lasted for at least 20 min. The level of LTP appeared to saturate by the second or third train set. Concurrent tetanization of both SD and PP fibres, however, resulted in a significant additional increment in the PP-GC population spike, but no further increase in the population EPSP. This additional increment in spike amplitude was more than would be predicted from the sum of the individual effects.

The interaction effect is probably due to a postsynaptic mechanism. There may, for example be neurochemical interactions triggered by the coactive inputs, or SD trains may interfere with the GC's recurrent inhibitory circuits and thereby increase the postsynaptic effect of each PP train. Whatever the mechanism, these 2 pathways are more distinct in terms of origin, termination zone and neurochemistry than other combinations that have been tested. These differences may facilitate investigation of the mechanisms underlying the cooperativity effect.

215.11 EFFECTS OF LONG-TERM TEMPERATURE ACCLIMATION UPON IONIC CURRENTS IN SNAIL NEURONS. Steven N. Treistman and Barry S. Layton. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We are examining the effects of long-term temperature acclimation upon the response of identified nerve cells in the land snail, Helix, to acute temperature change. Animals are kept in moist containers with lettuce and $CaCl_2$ at either 5° or 20° C for periods of 6-8 weeks. The presence of food and daily handling were found to prevent the onset of estivation. For experiments, nerve cell bodies are exposed by microdissection of the ganglion sheath, and selected cells are axotomized by undercutting, to maximize space clamp efficiency during voltage clamp steps. By use of pharmacological agents, we are able to measure a number of the ionic currents in these cells in relative isolation from each other. The following currents are being examined with respect to activation and inactivation characteristics and amplitude: 1) early transient inward sodium and calcium, 2) early transient outward potassium (I_A), 3) calcium-dependent potassium.

The data which we have collected to date indicate that the inward currents associated with the action potential are less affected by temperature history than are the currents which underlie the temporal patterning of spike output.

We will discuss these results with respect to the possible mechanisms of long-term neuronal adaptation: in particular, 1) alteration of membrane "fluidity" in the nerve cell membrane comparable to that seen in the membranes of simpler organisms during adaptation (with the possibility that specific lipid microdomains might be selectively altered) and 2) altered metabolic functioning which would affect calcium-dependent currents. Supported by grant BNS 81-09348.

215.12 DIFFERENTIAL AGING OF MOTOR NEURONS INNERVATING THE SAME ORGAN IN APLYSIA. B. Peretz. Dept. of Physiology and Biophysics, Univ. of Ky., Lexington, Ky. 40536.

Muscle contraction and junctional transmission elicited by L_7 at its terminals in the gill decrease significantly with age. Comparison between mature, average post-metamorphic age (PMA)-120 days, and old animals, PMA-200 days, showed that facilitation to spike trains was less in old animals. We concluded that old terminals could not attain or sustain the level of facilitation to trains of that measured at mature terminals, and that it resulted in decreased antiflaring contraction of gill pinnules (Peretz, et al. J. Neurobiol. 1982).

The study was extended to another motorneuron, LDG_1, to determine if age-dependent changes of function occurred uniformly for neurons innervating the gill. Somata of gill motor neurons are located in the parieto-visceral ganglion, abdominal ganglion. LDG_1 spiking elicited contraction of the efferent vessel, which results in the movement of blood from the gill toward the anterior chamber of the heart. Junction potentials from the longitudinal muscles in the efferent vessel and muscle contraction were measured during LDG_1 spike trains; the same procedures used to measure the effect of L_7 spiking in the pinnule. The comparison between mature and old LDG_1 function revealed no significant differences in the two measurements. That is, in old and mature gills, facilitation to spike trains was similar as was efferent vessel contraction.

Differences between L_7 and LDG_1 are being sought, which could account for the age-changes in the former and not in the latter. (1) When the function of L_7 and LDG_1 was measured in young Aplysia, PMA-60 days, that of L_7 was somewhat higher than measurements in mature animals, whereas that of LDG_1 was comparable to measurements in both mature and old animals. (2) During spike trains eliciting muscle contraction, facilitation at L_7's junctions, which was greater and of longer duration than that at LDG_1 junctions, decreased with age; that at LDG_1 junctions remained unchanged. (3) Input resistance of L_7 was decreased significantly from young to mature to old, and the decrease reduced synaptic input to L_7 (Rattan and Peretz, 1981); that of LDG_1 appeared to remain unchanged in the three age groups. Is differential aging of L_7 and LDG_1 the result of altered activation of L_7 and in turn altered facilitation at its terminals as compared to the results with LDG_1? That is, is it altered interaction between extra- and intracellular mechanisms that leads to changes of function with age? The results show that age-related changes in neural function do not occur uniformly for neurons of similar function. (NIA and NIMH).

215.13 CHANGES IN THE ELECTRICAL PROPERTIES OF CAT SPINAL MOTOR NEURONS AT 7 DAYS AFTER AXON SECTION. E.D. Hall, Program in Pharmacology, Northeastern Ohio Univs. Coll. of Med., Rootstown, Ohio 44272.

In this study, the electrical properties of randomly sampled 7 day ventral root-sectioned spinal motor neurons were examined in comparison to normal cells in a separate group of unanesthetized C-1 sectioned cats using conventional microelectrode techniques (5-10 megohm; 2M K-Acetate). The purpose was to compare the early effects of axon section in unanesthetized animals with results obtained by other investigators mainly at longer times after axon section in pentobarbital anesthetized cats.

No effect of axon section was found in the resting membrane potential (65.1 ± 0.9 mV (S.E.) for 57 normal motor neurons vs. 64.1 ± 1.3 for 28 axon-sectioned cells). However, an examination of the characteristics of antidromic action potentials revealed a significant ($p<0.01$) 28% decrease in initial axon segment (IS) conduction time; a 38% decrease in the threshold for soma-dendritic (S-D) invasion and a 24.6% increase in the rate of S-D depolarization. These findings are qualitatively consistant with results obtained by others mainly at longer times after axon section. On the other hand, myelinated axonal conduction velocity and the S-D zero overshoot were not affected at 7 days as reported at later times. Another observation not previously reported was an increase in the S-D action potential after-hyperpolarization. Soma-dendritic refractoriness, as tested by paired antidromic pulses at a 2.5 msec interval was unaltered, but there was a lessened ability to differentiate the IS and S-D portions of the second action potential. The excitability of the 7 day axon-sectioned motor neurons was dramatically increased as judged by a 61.2% decrease in the rheobasic current as well as a striking increase in the slope of the current-frequency relationship for repetitive discharge in response to constant current injection. In conjunction with the increased excitability, a significant ($p<0.0001$) increase in the input resistance was observed (as determined by the antidromic spike method) from 1.3 ± 0.3 megohms in the normal motor neuron population to 3.7 ± 0.9 in the 7 day axon-sectioned sample. Other studies in pentobarbital anesthetized animals have shown either a lesser increase or no change in input resistance.

The current results suggest that some of the early retrograde neurophysiological effects of axon section are either greater than, lesser than, or different from, those measured previously in motor neurons that had sustained axon section for the most part at longer times prior to testing in barbiturate anesthetized animals. The structural and functional implications of the altered electrical properties in axon-sectioned motor neurons will be presented.

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215.14 PROLIFERATION OF ^{3}H-SPIROPERIDOL BINDING SITES DURING BEHAVIORAL RECOVERY FOLLOWING DOPAMINE SYSTEM DAMAGE. Kim A. Neve, Michael R. Kozlowski, Paul F. Stemmer* and John F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Damage to the mesotelencephalic dopamine (DA) system produces sensorimotor and ingestive impairments from which rats frequently recover. We have recently demonstrated that the time of onset of the lesion-induced enhancement of ^{3}H-spiroperidol binding is remarkably similar to that of recovery of somatosensory localization (Neve et al., Brain Res., in press). Supporting these findings were preliminary data from our modification of the in vivo binding method, in which ^{3}H-spiroperidol is diluted with 0.5 ml of a saturating (80 µM) concentration of unlabeled spiroperidol prior to the intravenous injection. We previously compared the time course of behvioral recovery to that of the proliferation of ^{3}H-spiroperidol binding sites in rats that did not recover. We now report that receptor proliferation occurs in recovering rats. We also present additional data to validate the use of the in vivo binding method in measuring lesion-induced receptor changes.

Male Sprague-Dawley rats were given ventral tegmental injections of 6-hydroxydopamine. Rats were tsted postoperatively for their ability to orient to somatosensory stimulation. ^{3}H-Spiroperidol binding was measured in recovering animals 3, 7, 14 and 60 d postoperatively. Rats under light ether anesthesia were given 15 µCi of ^{3}H-spiroperidol (27.6 Ci/mmol) diluted with 80 µM spiroperidol, injected in a volume of 0.5 ml into the penis vein. Most rats were sacrificed 1 h later, and the striatal and cerebellar radioactivity determined. Specific binding was determined by subtracting the binding in the cerebellar cortex from that occurring in each striatum. Six rats were treated with haloperidol (5 mg/kg, i.p.) 1 h prior to the ^{3}H-spiroperidol injection. Five other rats were sacrificed 2 m after treatment with ^{3}H-spiroperidol.

Recovering animals showed a rapid postoperative proliferation of neostriatal ^{3}H-spiroperidol binding sites in the lesioned hemisphere. By 14 d postoperatively, the denervation-induced increase (17.9 ± 6.00%) is similar in magnitude to that in rats that have not recovered. The hemispheric asymmetries were abolished by prior treatment with haloperidol (L/R x 100 = 105.5 ± 13.11%). In addition, the concentration of ^{3}H-spiroperidol in the neostriatum was not enhanced in the lesioned hemisphere at 2 m (107.6 ± 9.06%). These results indicate that the lesion-induced increase in neostriatal ^{3}H-spiroperidol retention reflects a proliferation of specific binding sites rather than enhanced entry of the drug into the denervated striatum. Increased receptor density may contribute to behavioral recovery by compensating for the decreased DAergic innervation of the neostriatum.

215.15 INTRAVENTRICULAR 6-HYDROXYDOPAMINE TREATMENT IN RATS PRODUCES A GRADIENT OF DOPAMINE DEPLETIONS WITHIN THE STRIATUM. S. Onn, M.J. Zigmond, E.M. Stricker and T.W. Berger. Departments of Biological Sciences and Psychol., Univ. of Pittsburgh, Pittsburgh, PA 15260.

After subtotal destruction of dopaminergic terminals in the rat brain by the neurotoxin, 6-hydroxydopamine (6HDA), we have observed an increase in the capacity of residual terminals to synthesize and release more transmitter. However, thus far all results have been derived from biochemical analyses using the entire striatum, which contains both lesioned and unlesioned sub-areas. In order to define more precisely the field of 6HDA-induced damage and the compensatory function of the residual terminals, we have employed three histochemical techniques: FITC-TH immunocytochemistry, PAP-TH immunocytochemistry and glyoxylic acid-induced histofluorescence (SPG). A biochemical analysis (HPLC with electrochemical detection) is then conducted on tissue-punched samples from each region, which has been visualized histologically with the SPG technique.

The results indicate that regions representing various degrees of dopaminergic terminal destruction occur within the striatum of adult rats after 6HDA treatment. At 7 days postlesion (250 µg 6HDA ivt, 30 min after 25 mg/kg desmethylimipramine, ip), all three histochemical techniques show few residual terminals in the most medial area of the striatum but the terminal density increases gradually toward the lateral area. Such a gradient is confirmed with MPV-2 photometry. The biochemical analysis also indicates a similar distribution of dopamine (DA) in residual terminals: approximately 2% and 60% of the control amount in the medial and lateral areas, respectively. On the other hand, the metabolic activity (as measured by the ratio of dihydroxyphenylacetic acid to DA) in the medial striatum (3.29) is much higher than that observed in the lateral striatum (0.24), which falls in the range (0.22-0.34) found in the intact control striatum. This higher metabolic activity suggests that an adaptive compensatory release of DA from residual terminals in the most heavily denervated area has occurred. However, at 40 days postlesion, DA levels in the medial area are increased to 10% of control values and its metabolic activity approaches the normal range. Additionally, histochemical evidence of collateral sprouting has been observed in the medial striata at 55 days postlesion. These successive biochemical and morphological changes may contribute to the recovery of function observed after 6HDA treatment in rats. We believe that the use of biochemical analysis performed in association with histological techniques in the same brain will provide a useful approach in our attempts to identify the biological events which follow subtotal destruction of central dopaminergic neurons.

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215.16 DO JUXTAGLOMERULAR CELLS DIE AFTER PERIPHERAL DEAFFERENTATION OF THE RAT OLFACTORY BULB? F. L. Margolis, H. Baker, T. Kawano* V. Albert*, T. H. Joh , Roche-RIMB, Nutley, N. J. 07110 and Lab. of Neurobiology, Cornell Univ. Med. College, New York, N. Y. 10021

Peripheral deafferentation of the rodent olfactory bulb results in the loss of dopamine (DA) content, tyrosine hydroxylase (TH) activity and immunocytochemical staining for TH in the juxtaglomerular (jg) cells. Reinnervation of the bulb by the afferent neurons results in the return of these parameters to control values. (Nadi et. al. Br. Res. 213, 365 (1981), Kawano & Margolis J. Neurochem (in press), Baker et. al. submitted). Is this transsynaptic phenomenon mediated by the loss and reconstitution of the bulbar jg neuron population or do the afferent neurons regulate TH expression in a static jg cell population? To study this, we evaluated DOPA decarboxylase (DDC) following unilateral surgical lesion as another marker of DA neurons. It was essential to demonstrate the cellular localization of DDC since this enzyme is present in cerebral vessels as well as in fibers and terminals of serotonin and norepinephrine containing neurons. Adjacent histological sections of bulbs from unilaterally deafferented rats were stained with antisera to either DDC or TH. In the unlesioned bulb, similar numbers of jg neurons stained with TH (928±20) and DDC (951±8), in the lesioned bulb, many fewer neurons stained with TH (198±24) than with DDC (670±32). Thus, in the lesioned bulb, there was a deficit of TH positive cells (as we reported earlier) with three times as many neurons stained for DDC as TH. To demonstrate that the same population of cells stained with both antisera, a series of sections was first stained with DDC antiserum using 4-chloronaphthol as the chromogen. After photography and destaining, the sections were restained with TH antisera with DAB as the chromogen and rephotographed. Every cell in the jg region stained with TH also stained with DDC suggesting that those cells stained for DDC in the lesioned bulb were the same cells which had lost the ability to stain for TH. In vitro measurements of DDC activity confirmed the immunochemical findings. One month following unilateral deafferentation, DDC activity in the lesioned bulb expressed as pmol/min/bulb was 65% of control values (2230±200) and (3570±256) respectively. However, since the mass of the lesioned bulb was 65% of control, DDC activity expressed as pmoles/min/mg tissue was the same in lesioned (81±5) and control (84±5) bulbs. Eight months after lesion, DDC activity was still at the same level. The DDC activity measurements and immunocytochemistry argue for the continued presence, in the lesioned bulbs, of DDC containing, TH deficient, dopaminergic neurons. This supports our hypothesis that TH expression in a stable population of jg DA neurons is regulated transsynaptically by the primary afferent olfactory neurons. Thus, juxtaglomerular DA neurons do not die after deafferentation.

215.17 ELECTROPHYSIOLOGICAL EVIDENCE THAT HIPPOCAMPAL MOSSY FIBERS FORM AN ABERRANT RECURRENT EXCITATORY CIRCUIT AFTER KAINIC ACID LESION. David L. Tauck and J. Victor Nadler. Depts. Physiology and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Bilateral intraventricular injections of kainic acid (KA) in the rat preferentially destroy the CA3-CA4 cells of the hippocampus. This lesion deprives the dentate granule cells of both postsynaptic targets and associational-commissural innervation. The granule cell axons, or mossy fibers, respond by greatly expanding their normally very sparse recurrent excitatory projection to the inner third of the dentate molecular layer. We have used the hippocampal slice preparation to determine whether these aberrant connections are electrophysiological functional.

Hippocampal slices were prepared from adult male Sprague-Dawley rats two weeks after administration of KA intraventricularly (0.5-0.8 μg into each lateral ventricle over a 30-min period) or intravenously (11 mg/kg into the tail vein). Control animals received injections of vehicle alone. In slices from control rats, antidromic stimulation of the mossy fibers elicited a single population spike in the granular layer of the fascia dentata. When pairs of antidromic stimuli were delivered to the mossy fibers with interstimulus intervals of 30-80 ms, the response to the second stimulus of the pair differed little from the response to the first. Similar results were obtained in slices from KA-treated rats which lacked an expanded recurrent mossy fiber pathway, as judged by the Timm's sulfide silver stain. In contrast, antidromic stimulation of the mossy fibers in slices having a histologically-demonstrable recurrent mossy fiber pathway elicited two or three population spikes separated by about 4.4 ms. The second and third population spikes, and usually the first as well, exhibited paired pulse potentiation. All effects of the KA treatment were abolished by superfusion with Ca^{2+}-free medium containing 3.8 mM Mg^{2+}, but the initial antidromic spike was unaffected. This result suggests that repetitive firing and paired-pulse potentiation of the antidromic spike depended on synaptic transmission. The effects of KA treatment could not be accounted for simply by loss or depression of GABAergic inhibition, since they were not reproduced by superfusion of control slices with 100 μM bicuculline methiodide.

These results suggest that the mossy fiber collaterals which sprout after a KA lesion form a functional recurrent excitatory circuit. Most likely, this aberrant pathway further compromises hippocampal function that has already been disrupted by destruction of CA3-CA4 cells. (Supported by NIH grant NS 06233.)

215.18 THE LONG-TERM EFFECTS OF CLIMBING FIBER - DEAFFERENTATION OF BOTH ADULT AND WEANLING RATS. B.A. Flumerfelt and W.A. Anderson*, Department of Anatomy, University of Western Ontario, London, Canada.

The climbing fiber input to the cerebellar cortex of both adult and weanling rats was eliminated using electrolytic (dorsal and ventral parapharyngeal approaches) and chemical (3-acetylpyridine (3-AP)) lesions. A total loss of the climbing fiber input to the ansiform lobule was found following 3-AP treatment which suggests that the inferior olive is the sole source of the climbing fiber input. Animals deafferentated of their climbing fiber input as adults or weanlings were allowed to survive for 123-180 days and subsequently examined following Golgi-Cox impregnation or processing for electron microscopy.

Evidence is provided which suggests that basket axons, and in particular the parallel fiber system, possess some capabilities for sprouting following climbing fiber - deafferentation. An ectopic formation of dendritic spines occurred following climbing fiber - deafferentation of the weanling rat, but not the adult. Golgi-Cox and ultrastructural examinations have revealed, furthermore, that these ectopic spines were restricted largely to the distal portions of the smooth branches of the Purkinje cell dendritic tree and were synaptically innervated by either basket axons or the parallel fiber system. These Purkinje cells showed no signs of transneuronal degeneration. Climbing fiber - deafferentated adult Purkinje cells, however, underwent marked transneuronal degenerative changes and appeared to have lost the potential to form ectopic spines. The inability of the adult Purkinje cell to form ectopic spines in an attempt to replace the excitatory postsynaptic potential of the climbing fiber varicosity appears to be directly related to the Purkinje cell's subsequent transneuronal degeneration. Image analysis of Golgi impregnated Purkinje cells has indicated significant losses in both smooth branch and spiny branchlet numbers following deafferentation of the climbing fiber input in the adult. The loss of smooth branches and spiny branchlet numbers which occurred following climbing fiber - deafferentation of the weanling rat, on the other hand, appears to be the result of a reduction in postnatal branching, rather than transneuronal degeneration. In addition, transneuronal degeneration has been reported in the population of molecular layer interneurons following climbing fiber - deafferentation of both weanling and adult rats. These changes are evidence for a direct climbing fiber input to these cells.
(Supported by the MRC of Canada).

215.19 TRYPTOPHAN HYDROXYLASE IN THE HIPPOCAMPUS AND MIDBRAIN FOLLOWING 5,7-DHT INJECTIONS INTO THE CINGULUM BUNDLE. C.Clewans*and E.C.Azmitia (SPON: A.R. Eden). Dept. of Anatomy, Mt. Sinai School of Med. NY, NY 10029

Homotypic collateral sprouting of serotonergic (5-HT) fibers travelling in the fornix-fimbria (FF) has been shown to account for the anatomical and functional restoration of the 5-HT innervation of the rat hippocampus (HIP) following destruction of 5-HT fibers in the cingulum bundle (CB) (Azmitia et al., Nature 274: 374, 1978; Zhou and Azmitia, Soc.Neurosci.Abstr. Vol.7, p.68, 1981). Measurements of activity of the rate limiting enzyme in 5-HT synthesis, tryptophan hydroxylase (TPOH), support these earlier findings.

TPOH activity was assayed according to the method of Ichiyama et al. (J.Biol.Chem. 245:1699, 1970) in the presence of atmospheric oxygen, 80uM L-1-C^{14}-tryptophan, 116uM tetrahydrobiopterin, 1.0mM dithiothreitol, 14uM Fe^{++}, 0.4mM NADH, excess aromatic L-amino acid decarboxylase, and 0.1mM pyridoxal phosphate. Enzyme activity was measured in rat hippocampi after unilateral CB injections. Doses of 2, 3, 4, or 5 ug 5,7-DHT (free base) resulted in a similar decrease (-62%) in TPOH activity in the ipsilateral HIP 7 days after the injection. However, in the contralateral HIP, only the higher doses (4 and 5 ug) resulted in a significant (-39%) decrease. This latter result suggests that spread of the neurotoxin has occurred causing damage to the 5-HT innervation of the contralateral HIP.

Unilateral CB lesions with 5ug of 5,7-DHT caused a gradual reduction in TPOH activity of both ipsilateral and contralateral HIP reaching a maximum at 7 days (-62% and -48% respectively). There was a significant recovery of activity by 42 days after the lesion to -38% (ipsilateral) and -20% (contralateral). Experiments are in progress to determine whether this increase in TPOH activity at 42 days is the result of homotypic collateral sprouting of 5-HT fibers from the FF.

TPOH activity in the midbrain, the site of origin of the 5-HT fibers in the HIP, increased 21% above control by 42 days after the lesion, suggesting a compensatory increase in enzyme synthesis to accomodate newly sprouted 5-HT terminals in the HIP.

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215.20 HANDLING ENRICHMENT AND HIPPOCAMPAL PLASTICITY. M.D. Chafetz, D.L. Bernard*, E. Bergeron*, S. Wells*. Depts Psychology and Physics. Univ. of Southwestern Louisiana, Lafayette, LA 70504

The objective of this work is to identify one of the codes for enrichments effects on behavioral and neural plasticity. Enrichment is usually defined as increased sensory stimulation along one or more dimensions (Bennett, et.al., 1964). Handling therefore becomes somatosensory enrichment, yet the codes remain obscured by contradictory manipulations. Handling has been manipulated by amount (Seggie, J., 1968), and time (Coscina, D.V., et. al., 1975). In that frequency and population codes are characteristic of somatosensory receptors, it becomes important to distinguish the overall handling codes by independent manipulations. Because the hippocampus is a well known model for neuronal plasticity associated with enrichment, we used the Olton 8-arm maze as a behavioral test sensitive to changes in hippocampal function. Specifically, in Phase I, we sought to determine the effect each handling code had on 8-arm maze behavior, to identify which code was most important, and to determine which 8-arm maze behaviors were most closely associated with each code. In Phase II, we developed a heavy metals assay for changes in hippocampal function. Iron, zinc, and copper are all closely associated with enzymes that participate in neurotransmitter synthesis. Furthermore, zinc is a regulator of nerve growth factor sub-unit binding. Changes in hippocampal structure and/or function may therefore be associated with heavy metals effects.

Neither handling code has a simple, linear effect on behavior observed in the Olton 8-arm maze. The non-linear effect may be related to the time of day at which the animals are tested.

A heavy metals assay was developed using a particle-induced X-Ray emission analysis. Iron, zinc, and copper were identified and measured at their characteristic energy levels. The amounts of each metal corresponded to that reported by Kemp and Danscher (1979). Heavy metals levels were more closely associated with individual differences than with handling effects.

215.21 SPECIALIZED SYNAPSES AND DIRECT CELL-CELL APPOSITION IN RAT SUPRAOPTIC NUCLEUS: INCREASES WITH CHRONIC PHYSIOLOGICAL STIMULATION. Charles D. Tweedle and Glenn I. Hatton, Department of Anatomy and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Water deprivation for short periods of time (4-24 h), parturition, or 7-14 days of suckling have been shown to increase the amount of direct soma-somatic contact between magnocellular neurosecretory cells (MNC's) of the supraoptic nucleus (SON) (Cell Tiss. Res. 1977, 181, 59-72; Neurosci. 1981, 6, 919-930; Brain Res. Bull. 1982, 8, 197-204). This increased contact is apparently by means of the withdrawal of thin glial processes from between the MNC somata. Suckling (but not the other stimuli) also brings a dramatically higher incidence of specialized presynaptic terminals contacting more than one post-synaptic element (one MNC soma with another soma or a dendrite) in the same plane of section, a rare phenomenon in the SON of virgin female control animals. An experiment was designed to assess whether the increase in these specialized synapses could be brought about by chronic activation of the SON without the hormonal changes that accompany suckling.

Young adult (100 day old) virgin female rats were maintained for 10 days on 2% NaCl instead of drinking water to bring about an increase in SON MNC firing. Thin sections of SON from the saline-treated and control female rats (Holtzman strain) were quantitatively examined at the ultrastructural level. 3.5% of SON cells were in direct contact in the virgin female controls and 31.6% in the saline-treated animals, with less than .1% of total membrane in contact for the controls and 6.8% for the saline-treated group. 2.3% of MNCs showed specialized synapses in the SON of the control animals compared to 46.0% in the saline-treated animals. The % of cells showing "double" synapses with one presynaptic terminal onto two adjacent MNC somata was 8.8% in the experimental animals, with none seen in the control animals. Older (10 mos.) saline-treated female rats showed an incidence of 37.1% SON cells with specialized synapses, suggesting little effect of age on the synaptogenesis. The specialized synapses contain mainly 40-60 nm synaptic vesicles with a few 90-100 nm dense core vesicles and are not affected by 5-OH Dopamine treatment.

It thus appears that chronic physiological activation of MNCs either by suckling or saline treatment can induce the appearance of new non-catecholaminergic synapses in the SON, presumably as an adaptation to the stimulation. Where these synapses come from, how they form and what they do remain to be determined, as well as whether the stimulus for their formation is pre- or post-synaptic. (Supported by NIH Grant NS 09140.)

215.22 SYNAPTIC PLASTICITY OF AGING AT A MOUSE NEUROMUSCULAR JUNCTION: A SCANNING AND LIGHT MICROSCOPIC STUDY. M.A. Fahim, J.A. Holley* and N. Robbins. Anatomy Dept., Med. Sch., Case Western Reserve Univ., Cleveland, Ohio 44106

Ultrastructural study of aging neuromuscular junctions has already uncovered profound changes at aging mouse nerve-muscle junctions (Fahim & Robbins, J. Neurocytol., 1982 in press). In order to relate these findings to the overall structure and physiology of aging junctions, we have used a combination of scanning and light microscopy. All changes reported here were statistically significant. A combined silver-cholinesterase method was adopted for studying the number and length of nerve terminal branches in the extensor digitorum longus (EDL) of young (7 mo.) and old (29 mo.) mice. In old animals, there were increases in nerve terminal length (↑58%), number of intrasynaptic branches (↑87%) and endplate area (↑15%), with no change in muscle fibre diameter. However, extensive collateral sprouting or innervation, the hallmarks of partial denervation, were absent.

Scanning electron microscopy (SEM) of motor endplates was carried out by a modification of methods by Desaki & Uehara (J. Neurocytol., 10:101-110, 1981). Both young and old endplates appeared as slightly elevated, elliptical plateaux ("raised areas") with smooth surfaces into which the synaptic clefts were etched. In the aged endplates, the primary clefts were often interrupted by narrow outpouchings approx. perpendicular to the primary cleft long axis. Other differences between young and old endplates included: 1) an increased number of shorter primary grooves (79% had more than 3 primary cleft branches shorter than 2.5 μm compared to 21% in young endplates) and 2) an increased randomness of secondary fold orientation with age. Morphometric analysis of aged compared to young junctions revealed significant ($p<0.01$) increases in total groove length (↑26%), total groove perimeter (↑27%), groove area (↑23%), and the number of branches (↑111%) with age.

Thus, both light and scanning microscopy gave concordant evidence that nerve terminals and underlying postsynaptic clefts are more branched in aged mice. In aged animals, the previously reported diminution in nerve terminal cross-sectional area (Fahim & Robbins, op.cit.) combined with the extension in length of intrasynaptic nerve branches reported here, quantitatively supports the conclusion that nerve terminal volume is approximately preserved. The increased nerve branching at aged junctions may represent additional growth of functional nerve terminals within the junctional region, possibly compensating for reduced transmitter release per site by providing more release sites.

This work was supported by USPHS grant AG 00795.

215.23 A CONTINUOUS CONVERSION-DISASSEMBLY HYPOTHESIS FOR SYNPASE TURNOVER: A QUANTITATIVE SERIAL SECTION STUDY. S.F. Hoff and C.W. Cotman, Dept. Pharmacol., Chicago Med. School, North Chicago, IL 60064 and Dept. Psychobiology, Univ. California, Irvine, CA 92717.

After a unilateral lesion of the entorhinal cortex, one cycle of non-degenerative synapse turnover occurs within the non-denervated inner molecular layer of the ipsilateral dentate gyrus. We have observed in single montages a significant loss of simple synapses by two days post-lesion, with a subsequent, almost equal increase in complex (W and U shape) synapses during the 10 day post-lesion period, indicating a possible conversion of simple synapses to complex types. Also, the complex synapses have one or more perforations in the post-synaptic density (PSD), suggesting the disassembly process (Nieto-Sampedro, M., Hoff, S., Cotman, C., PNAS (1982) in press).

We have used quantitative serial section analysis and three dimensional reconstruction to evaluate the fate of the PSDs involved in the turnover cycle. Ninety day old Sprague-Dawley rats received unilateral lesions of the entorhinal cortex. After 10 days these animals and an unlesioned control group were sacrificed and prepared for electron microscopy.

Our results support a conversion-disassembly hypothesis for synapses undergoing turnover with the following data: (1) the relationship between PSD length and synaptic type (simple or complex) converges during the post-lesion time period. (2)linear regression analysis shows a significant correlation between PSD diameter and total perforation diameter up to a point. After the PSD diameter reaches 650 nm, the relationship becomes random. (3) the more complicated "W" type PSDs are significantly thinner at 10 days post-lesion. (4) Preliminary three dimensional reconstruction of PSDs from complex synapses do not demonstrate the expected disk shape for PSDs and their perforations, but instead a more tortuous shape with major open flaws in the PSD structure, many extending to the edge of the PSD.

Thus it appears that during the course of synapse turnover a simple synapse may: (1) increase the PSD diameter and thickness, (2) form a "U" shape with one or more central perforations, which become more extensive as the PSD continues to increase in diameter, (3) then form a "W" shape as the central perforation(s) enlarge, (4) undergo fragmentation of the PSD, and the material from them is redistributed for PSD assembly at new synaptic sites or is completely degraded. Thus a lesion-induced conversion-disassembly process provides one possible mechanism for synapse breakdown and redistribution of PSD material. This may resemble a slower on-going process of synapse turnover under normal conditions. Supported by NIMH grant MH19691 to CWC.

215.24 EVIDENCE FOR NATURALLY OCCURRING MOVEMENTS OF RETINOTECTAL TERMINALS IN GOLDFISH. S. S. Easter, Jr. and C. A. O. Stuermer*. Div. Biological Sciences, U. Michigan, Ann Arbor MI 48109.

In goldfish, retina and tectum grow concurrently, but in different patterns. Both add new neurons at their edges; in retina, around a complete ring, but in tectum around only about 2/3 of the perimeter. Thus one retinal region (temporal) produces new ganglion cells which project to a tectal region (rostral) where no new cells are added. The retinotopic projections are similar in both large and small fish. Roughly, the retinal center projects to tectal center, retinal edges project to tectal edges, and the tectal magnification factor (um on tectal surface/degree of visual angle) is constant in a given tectal lobe. How is this orderly projection maintained when the two interconnecting surfaces grow so differently? It has been suggested that the new axons from temporal retina displace old ones from their terminations on rostral tectum, that the displaced axons, in turn, displace others more caudally, and that this process of removing and forming terminals goes on continuously as a normal part of growth. These hypothetical "sliding terminals" are the subject of this abstract.

We have developed a model for retinotectal growth and connectivity which predicts the intratectal paths of retinal axons and terminals. The model is based on the following. 1) The ingrowing axon tips of the new ganglion cells enter tectum rostrally, advance along the dorsomedial or ventrolateral tectal boundaries and terminate nearby at the retinotopically correct site in peripheral tectum. The "primary trajectory" of a retinal axon tip is its course along the then tectal boundary to its original termination site. 2) As retina and tectum grow, the terminals already in tectum are displaced by new ones to positions which maintain the orderly retinotopic map. As a terminal moves, its axon elongates to keep the connection between the site of the original termination and the terminal at its current position. The elongated axon passes through all the sites where it had terminated previously, and so traces out the "secondary trajectory" of the growing axon tip. The primary and secondary trajectories are predicted by the model.

We have tested these predictions anatomically. HRP was inserted into various tectal sites, where it was picked up by both terminals and axons of passage in the vicinity and transported retrogradely to the somata of retinal ganglion cells. The retinas were then reacted with TMB or DAB and examined as whole mounts. The surprisingly complex patterns of labeled ganglion cells were inconsistent with previous suggestions that the optic fibers course directly toward their site of termination, but consistent with the predictions of the model.

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215.25 NEURONAL PLASTICITY IN THE HIPPOCAMPAL FORMATION OF THE ADULT MONKEY FOLLOWING LESIONS OF THE ENTORHINAL AREA. M. Moss, D.L. Rosene and G.W. Van Hoesen. Harvard Neurology Unit, Beth Israel Hosp., Boston, MA; Dept. of Anatomy, Boston Univ. School of Medicine, Boston, MA and Dept. of Anatomy, Univ. of Iowa, Iowa City, IA.

It is now well established that in rodents, partial deafferentation of the hippocampus causes this structure to undergo extensive neuronal reorganization, both in the neonate and adult animal. We can now report anatomical evidence of changes in normal hippocampal morphological organization in response to ablations of the entorhinal area in the adult primate. In five normal monkeys (four Macaca fascicularis and one Macaca mulatta), the entorhinal area, including the peri- and prorhinal cortices, was removed in the left hemisphere. Survival times were one, three, eight, ten and seventeen weeks. In addition, the entorhinal area was removed unilaterally in a rhesus monkey with a chronic bilateral fornix transection and was allowed to survive for thirteen weeks. The brains were processed with the Karnovsky-Roots procedure for identification of acetylcholinesterase (AChE) and the reaction product was quantified using a scanning and integrating microdensitometer. Measurements were made in all layers of hippocampal sectors CA3, CA1 and the dentate gyrus as well as in all sectors of the subicular complex at four rostro-caudal levels. AChE levels were expressed as a percentage of AChE densities in the ipsilateral caudate nucleus. No appreciable changes in AChE levels in any part of the hippocampal complex were found in the animals which survived one or three weeks. In sharp contrast, substantial changes in AChE density were observed in the animals which survived either eight, ten or seventeen weeks. Increases in AChE density were found in both the middle and outer thirds of the molecular layer of the dentate gyrus to approximately the same extent in all three cases. The response was greatest in the most rostral portion of the hippocampus with increases in density ranging from 20% -50% of the homotypic sites in the contralateral normal hemisphere. No consistent changes were found in any other regions of the hippocampus or subicular complex.

The one animal which sustained removal of the entorhinal area after chronic fornix transection showed no increase in AChE density but, rather, showed a substantial loss throughout most of the hippocampus, including the molecular layer of the dentate gyrus. The findings suggest that 1) The primate hippocampus has the capacity for neuronal plasticity in the adult as well as in the neonate animal (Moss, Rosene and Mahut, 1982). 2) The response after entorhinal ablation may be dependent on sprouting of the intact cholinergic input from the septum via the fornix into the adjacent deafferented dendritic fields. 3) Both the appearance and completion of the response after deafferentation occur later than in the rat. Supported by Grant Nos. NSF BS 7717212, BNS 7924099, NS 14944 and NIA AG00001.

215.26 AUTORADIOGRAPHIC TRACING OF A CROSSED CORTICO-RUBRAL PATHWAY IN CATS WITH NEONATAL ABLATION OF ONE CEREBRAL HEMISPHERE. B. J. Sonnier, J. R. Villablanca, Ch. Olmstead, J. P. McAllister and F. Gomez. Ment. Ret. Res. Ctr., Depts. Anat., Psychiat., UCLA Sch. Med., Los Angeles, CA 90024.

The left half of the telencephalon was removed (hemispherectomy) in 6 kittens ($\bar{x}$ age 12.3 + 7.6 days), their neurology was followed into adulthood (companion Abstract) and they were used for this study at $\bar{x}$ age 536 + 34.9 days. The cats received injections of a 50:50 mixture of tritiated leucine-proline into the right pericruciate cortex. Starting at about 4 mm from the midline, 5 to 6 injections were made (0.2 to 0.6 μl at 50 μ Ci/μl) 1.0 to 1.5 mm in front and behind the cruciate at a 3 mm depth. Five days later cats were perfused intracardially, frozen coronal sections were cut at 50 μm and processes for autoradiography (method of Cowan, et al). Sections were developed after a 6 week exposure and injection sites and terminal fields were reconstructed from drawings made under both light and dark field illumination (atlases of Berman and Reinoso-Suarez). The outstanding finding was the presence of terminal fields in the red nucleus (RN) of both sides. Left RN terminals appeared to originate from labeled fibers that ran medially from the right mesencephalic penduncle and coursed toward the midline either through or under the right RN. Preliminary evaluation of the topography of right and left RN terminal fields showed: 1) in 4 cats terminal density was similar in both nuclei, while in other 2 terminal labeling was sparser in the left RN; 2) terminal labeling was seen along the entire A-P extent of the nuclei except in 2 cats where the caudal 1/3 were devoid of labeling, but in all cases there was an area of increased density which tended to be located in the anterior half; 3) most terminal label was confined to the ventral half or 2/3 of the RN; 4) terminal fields tended to cluster toward the medial half of the nuclei in 3 brains while in other 3 the lateral-medial distribution was variable. Therefore, the distribution of terminal fields was similar for both RN in each hemispherectomized cat and the topography of these endings was also basically similar to that in our intact cats (companion Abstracts) except for a possible rostral displacement of the fields and more variability in hemispherectomized animals. The literature, as well as our own results in intact animals, indicate that cortico-rubral projections are normally ipsilateral in cats. In addition, we found no difference in the injection sites of our intact versus hemispherectomized cats which could account for contralateral RN labeling in lesioned cats. Although further quantification is needed, the present finding and additional reports here and in Neurosci. Lett., 29: 25; 82, indicate that after hemispherectomy in cats there is anatomical reorganization of the brain stem and that this might be more extensive if the lesion is inflicted neonatally. USPHS Grants HD 04612 and HD 05958.

215.27 DIFFERENTIAL RECOVERY FROM HEMISPHERECTOMY IN KITTENS AND CATS. J. W. Burgess, J. R. Villablanca, Ch. E. Olmstead and M.S. Levine. Mental Retardation Research Center, Depts. Anatomy and Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.

The left half of the telencephalon was removed (hemispherectomy) in 6 kittens (HEMI K, $\bar{x}$ surgery age 12.3 + 7.6 days) and 10 adult cats (HEMI C). Neurobehavioral effects were monitored into adulthood ($\bar{x}$ age 536 + 34.9 days) for HEMI K and at least 6 mos. for HEMI C. All HEMI K showed normal weight gain. Comparisons in a battery of neurobehavioral tests at 6 months postsurgery or later showed that: In spontaneous walking paresis of contralateral limbs was absent in HEMI K but noticeable in HEMI C ($p<.001$); similarly, when pulled forward, resistance with contralateral forepaw was stronger in HEMI K (abduction angle $\leq 45°$) than in HEMI C (abduction $\leq 90°$). The contralateral forepaw was usually extended with mild to moderate hypertonia in HEMI C while there was tendency to hypotonia with irregular posture in HEMI K. Further sign of paresis in HEMI C but not in HEMI K ($p<.001$) was a larger right eyelid opening. Chin and visual placing of the contralateral forepaw were absent and proprioceptive placing of that rear limb was absent or of very high threshold in HEMI C with severe impairments in narrow plank-walking. In contrast, these reactions were bilaterally present in HEMI K ($p<.005$) and only showed higher threshold and/or were slower in the right side such that plank-walking was easier ($p<.005$). In a string-toy test for paw-usage (up to 3 months of age) HEMI K showed no significant asymmetry whereas HEMI C showed bias for left paw ($p<.02$); similarly, when food was easy to get only with the impaired paw, HEMI K were proficient in one session while HEMI C required at least 1 month postsurgery, several sessions and partial restriction of left paw. If food was obtainable with either paw, HEMI-C rarely reached with impaired paw whereas HEMI-K did reach with that paw in early sessions but declined thereafter. In an open-field, HEMI K showed only slight bias for turning ipsilateral to the ablation while HEMI C turned exclusively towards that side. In a modified T-maze with reversal training both HEMI groups performed much worse than intact cats (95% correct) and HEMI C made fewer ($p<.05$) correct responsed (52%) than HEMI K (70%). Additional impairments were absence of contralateral contact placing, marked decrease in vision with the contralateral eye (absence of visuo palpebral reflex) and decreased somatonsensory reactivity. Subjectively, these also appeared to be less marked in HEMI K. Anatomically, lesions were comparable in both age groups. In conclusion, there were extensive recoveries in both HEMI groups but these were significantly larger in neonatally lesioned animals. We are now attempting to correlate the recoveries with possible brainstem reorganization (see companion Abstracts). USPHS Grants HD-05958, and HD-04612.

215.28 AUTORADIOGRAPHIC TRACING OF THE CORTICO-RUBRAL TERMINAL FIELDS IN CATS. J.P. McAllister, J.R. Villablanca, Ch.E. Olmstead, F. Gomez, and B.J. Sonnier. Ment. Ret. Res. Ctr., Depts. Anatomy and Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.

The existence of a projection pathway from the pericruciate cortex to the ipsilateral red nucleus in cats is well documented, however, there is no agreement regarding the distribution of the terminal field. While some authors claim that the projections are mostly limited to certain areas of the nucleus, others maintain that they are homogenously distributed. None of the studies that we know of (except for a brief account in J. Hirnforsch., 20: 375; 79) have applied the autoradiographic tracing of axons to this problem although it has some advantages over traditional silver impregnation methods. In addition, we needed this baseline study for comparisons with our brain-lesioned cats (companion Abstracts). Adult cats (N=10), 3 of which were reared in our Kitten Colony, received injections of 50:50 mixture of tritiated leucine-proline into the right pericruciate cortex. Starting at about 4mm from midline, 5 to 6 injections were made (0.2 to 0.6 μl at 50 μCi.μl) 1.0 to 1.5 mm in front and behind the cruciate sulcus at depth of 3 mm. Five days later the cats were perfused intracardially, frozen sections were cut coronally at 50 μm and processed for autoradiography (method of Cowan, et al.). Sections were developed after a 6 week exposure and injection sites and terminal field were reconstructed by serial drawings under both light and dark field illumination (atlases of Berman and Reinoso-Suarez). In all brains terminal fields were seen only in RN ipsilateral to the injection site. There was some terminal labeling along the entire A - P extent of the nuclei in all cases. In 7 brains there was an area of increased grain density located caudally in the middle 1/3 of the nucleus. Fibers contributing to these fields ran medially from the right mesencephalic peduncle and reached the nucleus sparsely and diffusely at all A - P levels. In most brains terminal fields were confined to the ventral half or 2/3 of the nucleus and in all but 2 brains, grains tended to cluster in the medial half of the RN. At the site of the injections the radioactive material usually filled the three sigmoid gyri but excluded the medial 1/4 of the anterior and posterior gyri. In 4 cases these medial areas were also partially labeled including portions of gyri located in the medial surface of the hemisphere. Our results confirm that the cortico-rubral projection from dorsal pericruciate area is ipsilateral in cats. They also suggest that although there are terminals along the entire A - P extent of the RN they are more dense at level of the middle 1/3 of the rostral-caudal extent and towards ventral and medial regions of the overall nucleus.

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215.29 REORGANIZATION OF CEREBELLORUBRAL TERMINAL FIELDS FOLLOWING HEMISPHERECTOMY IN ADULT CATS. Ch.E. Olmstead, J.R. Villablanca, J.P. McAllister, II, B.J. Sonnier, and F. Gomez. Ment. Ret. Res. Ctr., Depts. Psychiat. and Anat., UCLA Sch. Med., LA, CA 90024

As part of a continuing study of recovery of function in animals with uni- and bilateral removals of the cerebral hemispheres, we are examining possible sites for interactive remodeling in areas where cortical and cerebellar projections overlap (Neurosci. Lett., 29:25, 1982 and companion abstracts). We report here results from injections of tritiated amino acids into the nucleus interpositus posterior (INP) and the subsequent mapping of projections to the red nucleus (RN). Intact (N=7) and left hemispherectomized (HEMI:N=8) adult cats received single unilateral injections (0.2 µl at 50 µCI/µl), of a 50:50 mixture of tritiated leucine-proline into INP on the right side. Five days after the injections the animals were perfused intracardially with 10% neutral formalin and frozen sections were processed for routine autoradiography. Injection sites and terminal fields were reconstructed under both light and dark field illumination. 1) In intact cats, following injections into the INP, dense terminal label was discretely confined to caudal, medial and ventral regions of the contralateral RN. This area was populated predominantly by large neurons. More rostral portions of RN that contained smaller neurons were either sparsely labelled or devoid of terminal label. Labelled fibers which continued on to the thalamus could be seen coursing linearly in a caudal to rostral direction through the entire RN. 2) The most striking feature in all of the HEMI cats was that the terminal labelling moved rostrally, laterally and dorsally to occupy an area comprised of small and medium sized rubral neurons. In all cases the label appeared as a more dense combination of both terminals and fibers than that seen in the intact subjects. The relative size of the terminal field apparently was not different from that seen in intact cats with an identical injection. In addition to the ascending cerebellothalamic fibers which coursed linearly through the nucleus, there were numerous other fibers that often ran obliquely within the RN or arched around the border of the terminal field. This profusion of fibers was not seen in the intact cats. Our results suggest that any reorganization of cerebellar terminals to occupy sites vacated by cortical afferents may involve concomitant degeneration of cerebellar terminals or retraction from their original location. Further, the cerebellar projection does not appear to enlarge its terminal field after cortical lesions, rather it seems to shift to a new location within the red nucleus. Since the dendrites of the large rubral neurons are extremely long it is possible that the cerebellar terminals have rearranged themselves onto the more distal portions of the same neuron. USPHS Grants HD-04612, HD-05958 and HD 07032.

215.30 ACUTE INFRAORBITAL DEAFFERENTATION ALTERS RECEPTIVE FIELDS OF SOMATOSENSORY NEURONS IN HAMSTER SUPERIOR COLLICULUS. R.D. Mooney, R.W. Rhoades, and M.F. Jacquin. Dept. of Anatomy, N.J. School of Osteopathic Med. and Rutgers Med. School, Piscataway, N.J. 08854.

In preparation for experiments in which the effects of neonatal infraorbital nerve section upon collicular somatosensory topography will be assessed, we have examined the extent to which such damage alters the organization of tectal receptive fields (RF's) when the lesion is carried out during the course of a recording experiment. In 10 animals, a penetration was made through the part of the colliculus in which the peripheral distribution of the infraorbital nerve (ie. the vibrissae follicles, common fur of the whisker pad, upper lip and rhinarium) is represented. The RF for each cell encountered was carefully plotted. At the bottom of the track a unit with an RF restricted to infraorbital territory was isolated and the nerve was sectioned intraorbitally. In 7 animals the same cell was recorded before and after the nerve cut. In all instances the infraorbital RF was immediately lost and over a short period (1-45 min) the cell became responsive to other parts of the contralateral and/or ipsilateral body surface. In all 10 animals, similar differences were noted between RF's for cells recorded before the cut and those for units isolated after the section, as the electrode was withdrawn.

In 8 additional experiments, subcutaneous injections of xylocaine were made into the RF's of 15 tectal cells. Such injections abolished responsivity to stimulation in the anesthetized infraorbital region in all cases (N=15). In 13 units responsivity to new portions of the body surface was noted. These "unmasked" RF's appeared gradually over a period of 5-20 min. In 9 cells a complete or partial return of the original RF and disappearance of the unmasked field was observed within 2 hr of the xylocaine injection. In the remaining 4 units, the RF was altered for as long (up to 3 hr) as the cell was recorded.

Additional control experiments were carried out in 5 hamsters to determine whether or not slight variations in levels of general anesthesia or nonspecific trauma associated with the nerve sections or xylocaine injections might be responsible for the changes observed. Neither "sham" nerve cuts, nor saline injections altered collicular receptive fields. Large increases in the depth of sodium pentobarbital induced general anesthesia did reduce spontaneous activity and cause a slight contraction of RF borders. Such changes were, however, insufficient to account for the effects produced by the infraorbital nerve cuts or xylocaine injections.

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215.31 CROSS-STRAIN TRANSPLANTS OF EMBRYONIC SEPTUM: SURVIVAL AND SPECIFICITY OF INNERVATION. Walter C. Low, Peter R. Lewis* and S.T. Bunch*, University of Vermont, Burlington, VT 05405 and University of Cambridge, Cambridge, U.K.

Recent anatomical studies using homogenic neural transplants of embryonic septum have demonstrated that the transplatns exhibit the capability to innervate the brain of adult host recipients. The reinnervation, furthermore, appears to be quite functional in terms of the physiology of synaptic connectivity and the behavior of the recipient animals. We now report that cross septal transplants between strains of rats that exhibit major histocompatibility differences are capable of surviving for extended periods of time without rejection. The reinnervation of the host hippocampus by the cross-transplanted septum was also found to be quite specific and mimicked the pattern of innervation displayed by homogenic transplants and by intrinsic septal projections.

Donor tissue was taken from a Sprague-Dawley (SD) strain of rat which exhibits an Ag-B6 histocompatibility haplotype and transplanted to adult Wistar rats that exhibit an Ag-B2 haplotype. Embryonic septum was dissected from 15-17 day old fetal pups and transplanted to the vasculature overlying the anterior thalamus. This vasculature was exposed when suction lesions of the fimbria-fornix were made to eliminate the intrinsic septal projection to the hippocampal formation. Three months after transplantation, animals were sacrificed for acetylcholinesterase (AChE) histochemistry and staining with cresyl violet.

Robust transplants were found in four of five animals studied. In sections stained with cresyl violet, the transplants exhibited regions containing an abundance of neurons which were normal in appearance. These regions were essentially free of lymphocytic infiltration. In other localized regions of the transplant, mast cells, lymphocytes and enociphiles were observed to be diffusely scattered and was suggestive of a continuing immune response.

Hippocampal sections stained for AChE revealed a laminar pattern of staining typical of the intrinsic septal innervation of the hippocampal formation. Heavy staining was observed in the hilus, infra and supra granular layers along with diffuse staining in the outer molecular layer of the dentate. The inner molecular layer of the dentate was sparsely stained. In the regions of Cornu Ammonis, the infra and supra pyramidal cell layers were found to be heavily stained.

The results of this cross-strain study are in support of the privileged nature of embryonic tissue as a source of material for transplantation and of the privileged nature of brain as a site of transplantation. With regard to synaptic specificity, the appropriate pattern of cholinergic staining suggests that the mechanisms which regulate the specificity of fiber innervation and synapse formation are similar in SD and Wistar rats.

215.32 INTEGRATION OF CHICK NEURAL PLATE TRANSPLANTED TO THE CNS OF NEONATAL AND ADULT HOSTS. H. Jackson and F.A. Haun. Depts. of Neurosurgery, Anatomy and Otolaryngology, Univ. of Virginia Medical Center, Charlottesville, VA 22908.

In most instances of neural tissue transplants to the CNS of neonatal or adult hosts, the donor tissue is derived from the mid-term embryonic nervous system. It seemed possible that much younger donor tissue might 1) survive intraparenchymal transplantation better than older tissue, and 2) integrate with the host tissue to a greater degree due to the mitotic and migratory potential of the germinal cells and their progeny. Finally, there is reason to believe that the development of very young donor tissue might be influenced to a greater extent by the transplant environment. As a first stage in experimentation with transplants of neural plate cells, we examined their viability and general development. Chick embryos were labeled with ^{3}H-thymidine at 37 hrs of incubation. At 44 hrs, posterior trunk segments were dissected and trypsinized. The neural plate was then dissociated from surrounding tissues by pipetting in MEM with 15% horse serum. Plate segments were rinsed in Howard's saline and cut into small (~150 µm) pieces. One to three of these pieces were injected into the hyperstriatum of host chickens using a syringe with a bevelled glass needle. Twelve hatchlings (2 day) and three adult (3 mo) chickens received transplants and were killed at survival times of 2 days to 6 weeks. No sign of degeneration was seen in any of the transplants regardless of survival time. To establish whether donor cells were dividing in the host, a neonatal host was injected with ^{3}H-thymidine 12 hrs after receiving an unlabeled transplant, and was then killed 36 hrs later. Heavily labeled cells were found in and surrounding the transplant, indicating that the neural plate cells do indeed divide after transplantation.

Large numbers of labeled (i.e. transplant-derived) neurons were seen in the 2 neonatal and 1 adult host allowed to survive for 6 weeks. Light- and electron-microscopy showed that these neurons issued axons and dendrites and received synaptic contacts. The gross organization of these cells could not be distinguished from that of the host tissue. In fact, there was considerable intermingling of transplant-derived and host neurons; isolated labeled neurons were seen as far as 400 µm from the principal concentration of transplant-derived cells. To our knowledge, this is the first report of successful transplantation of neural plate cells to the CNS of neonatal or adult hosts. The initial findings suggest that transplantation of such tissue may lead to considerable integration of transplant-derived and host cells. This possibility and the nature of synaptic connections formed by transplant-derived cells are currently being studied in more detail.

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215.33 CROSS-SPECIES NEURAL GRAFTING: SURVIVAL AND FUNCTION IN AN ANIMAL MODEL OF PARKINSON'S DISEASE. U. Stenevi*, A. Björklund*, S.B. Dunnett* and F.H. Gage. Dept. of Histology, University of Lund, Lund, Sweden.

Previous studies from this laboratory have demonstrated that intracerebral implants of embryonic mesencephalic dopamine (DA) neurons, grafted between individuals of the same inbred rat strain, can reverse some of the functional deficits caused by damage to the nigro-striatal DA pathway. These observations have raised the possibility that the intracerebral neural grafting technique eventually may find a clinical application in the treatment of neurodegenerative disorders, particularly Parkinson's disease. One obvious obstacle for any such attempts is the immunological injection mechanisms associated with allogenic or xenogenic grafting. The brain has, however, been described as an immunologically "privileged" site, partly perhaps because of its protective blood-brain barrier.

In the present study we transplanted mesencephalic DA neurons, taken from albino mouse embryos (NMRI), to the dopaminergically denervated neostriatum in adult recipient Sprague-Dawley rats. The functional capacity of the grafted neurons was assessed by amphetamine and apomorphine rotation tests. Rotation tests were conducted at 1½, 3½ and 5½ months after transplantation. All animals were killed 6 months after transplantation and the brains were processed for catecholamine fluorescence histochemistry. The results demonstrate that long term survival of cross-species grafts of embryonic DA neurons can be achieved in the adult rat CNS in the absence of any immunosuppressive treatment. While the discrete grafts were largely resorbed, DA neurons had survived within the host striatum and given rise to a new DA innervation in the host striatum. The magnitude of DA fiber in growth into the host caudate-putamen and the degree of compensation of the turning asymmetri was well within the range of those seen with homologous nigral grafts. The conditions for functional transplantation of embryonic neurons across immunological barriers, to sites within the brain, thus seems to be more favourable than for peripheral grafts of neurons or endocrine cells, which are rejected unless heavy immunosuppressive treatments are applied.

215.34 FUNCTIONAL CHANGES FOLLOWING EMBRYONIC GRAFTS TO DEAFFERENTED HIPPOCAMPAL FORMATION. F.H. Gage, S.B. Dunnett*, A. Björklund* and U. Stenevi*. Dept of Histology, University of Lund, Lund, Sweden.

Complete bilateral fimbria-fornix transsection results in a 50% noradrenergic, 70% serotonergic and 95% cholinergic deafferentation of the hippocampal formation. In addition, this lesion induces a long lasting behavioral syndrome involving, hyperactivity, hyperreactivity and impairments in behavioral tasks associated with learning and memory. Preliminary studies from this laboratory have demonstrated that embryonic septal grafts placed in the fimbria-fornix lesion cavity can provide a cholinergic reinnervation of the denervated hippocampal formation and restore maze-learning capacity.

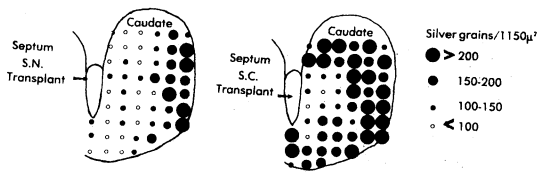
The present experiment was designed to provide a comparison of the ability for embryonic grafts rich in noradrenergic (locus coeruleus) cholinergic (septal) and serotoninergic (raphe) cells to ameliorate deficits associated with hippocampal deafferentation. Tests of functional recovery included open field activity, exploration, forced choice alternation, passive avoidance, spatial memory in a water maze, working memory in an 8-arm maze, and startle reactivity. Different grafts were effective in ameliorating different behavioral tests. Graft survival and extent of reinnervation was assessed in relationship to the extent of functional recovery. The results are discussed both in terms of the utility of embryonic neuronal grafting as a tool to understand basic mechanisms of brain function, and their utility to restore function disrupted by brain damage.

215.35 TISSUE TRANSPLANTS IN RAT BRAIN: ROTATIONAL BEHAVIOR AND DOPAMINE SUPERSENSITIVITY. G.N. Ko,[1] W.J. Freed,[1] D.L. Niehoff,[2] E. Cannon-Spoor,[1]* J.M. Morihisa,[1]* M.J. Kuhar,[2] B.J. Hoffer[3]* and R.J. Wyatt[1]. [1]Div. Spec. Ment. Res., NIMH; [2]Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Dept. of Pharmacol. Univ. Colorado Sch. of Med., Denver, CO.

Transplantation of fetal substantia nigra (SN) to the lateral ventricle, adjacent to the caudate nucleus, decreases lesion-induced rotational behavior (Freed et al. Ann. Neurol. 8:510-519, 1980). We have compared grafts of fetal SN with two other fetal brain areas, frontal cortex and superior colliculus (SC), to determine whether rotational behavior can be decreased by grafts of other tissues as well. Animals were lesioned, and after one month their rotational behavior in response to apomorphine was studied. Pieces of SN, frontal cortex, or SC were obtained from 17-day gestational rat fetuses and grafted to the right lateral ventricle. Two to three months later, SN grafts were found to have reduced rotational behavior by a mean of 30 ± 7 % (± S.E.M.: n=20; t=3.13, $p < 0.01$). Frontal cortex grafts did not reduce rotational behavior (3 ± 9 % reduction, n=9, t=0.74, $p > 0.4$). Grafts of SC did not significantly reduce rotational behavior, although there was a tendency in that direction (15 ± 8 % reduction, n=10, t=1.75, $p > 0.10$).

To determine whether the grafts reduced lesion-induced dopaminergic supersensitivity in the caudate nucleus, in vitro light microscopic autoradiography with ^{3}H-spiroperidol, according to the method of Polacios, Niehoff and Kuhar (Brain Res. 213:227-289, 1981) was performed on brain slices from animals with intraventricular grafts. There was a reduction in the density of silver grains in areas of the caudate adjacent to grafted SN. The same phenomenon was noted to a lesser extent in the caudate adjacent to an intraventricular SC graft.

In conclusion, the ability of fetal brain grafts to reduce rotational behavior appears to be specific to SN. The present findings suggest that such grafts reduce turning by releasing dopamine, thereby decreasing lesioned-induced supersensitivity in areas of the caudate nucleus adjacent to the grafts.



215.36 INTRASPINAL SPROUTING IN DORSAL ROOT AFFERENTS (DRAs) FOLLOWING PARTIAL SPINAL HEMISECTION: A RE-APPRAISAL WITH THE TRACER HORSERADISH PEROXIDASE. B. E. Rodin, S. Sampogna* and L. Kruger. Dept. of Anatomy, Brain Research Institute and Ahmanson Lab. of Neurobiology, UCLA, Los Angeles, CA 90024.

The evidence derived from silver studies in adult animals for sprouting in DRAs caudal to a spinal hemisection is difficult to interpret because denervation-induced changes in central nervous tissue, other than axonal growth, may alter silver impregnation patterns. In the present study, the question of intraspinal sprouting in DRAs following spinal hemisection was reexamined with an axonal labeling technique involving the transganglionic transport of horseradish peroxidase (HRP) from a peripheral nerve.

The central trajectory of sciatic afferents was compared in rats previously subjected to chronic (2-3 mos.) or acute (1 day) partial spinal hemisections (dorsal columns spared) at 30-120 days of age and in intact controls. Lesions were made at various levels (T9-L2) and sometimes included the corticospinal tract located in the ventral portion of the dorsal funiculus. At the prescribed time following surgery, the sciatic nerve on the lesioned side was exposed and sectioned in the upper thigh and dry HRP crystals were applied to its proximal cut-end. Animals were sacrificed five days later and the L4-L6 dorsal root ganglia, which give rise to the sciatic nerve in the rat, and spinal cords were removed and processed for visualization of HRP reaction product with tetramethyl benzidine as the substrate.

The pattern and density of the projection to spinal segments below the level of hemisection was not detectably different in animals with chronic or acute lesions and in intact controls. This was consistent even in areas for which anatomical and/or electrophysiological evidence for sprouting has previously been reported (medial and lateral base of the dorsal horn, intermediate zone, ventral horn) and when lesions included a portion of the corticospinal tract. It is concluded that axonal growth is lacking in DRAs following hemisection.

These results complement our previous finding that HRP-labeled DRAs do not form anomalous connections in the spinal cord after surrounding dorsal roots have been severed and cast serious doubt on the hypothesis that new axonal growth in DRAs can explain functional changes following partial denervation. Supported by NIH grant NS-5685.

215.37 EFFECT OF ADRENALECTOMY ON AXONAL SPROUTING OF HIPPOCAMPAL 5-HT FIBERS. F.C.Zhou* and E.C.Azmitia (SPON:J.E.Shriver). Dept. of Anatomy, Mount Sinai Sch. of Med. N.Y., N.Y. 10029.

Serotonergic fibers from the median raphe nucleus (MRN) use two pathways to innervate the hippocampus (HIP)--the cingulum bundle (CB) and the fornix-fimbria (FF) (Azmitia and Segal, J. Comp. Neurol., 179:641,1978). Selective lesioning of the CB 5-HT fibers induces homotypic collateral sprouting from FF 5-HT fibers which results in structural and functional recovery (Azmitia, Nature, 274;374, 1978; Zhou & Azmitia, Soc. Neurosci. Abstr. Vol. 7, p68, 1981). However, this compensatory growth in HIP is impaired by bilateral adrenalectomy (ADX).

30 female rats were separated into 5 groups: Normal control (n=5), sham injected control (n=6), ADX control (n=3), 5,7-dihydroxytryptamine (5,7-DHT, 4ug in 400nl ascorbic saline) microinjection into CB of normal (n=10), and 5,7-DHT microinjection into CB of ADX rats (n=6). Animals were microinjected with Horseradish peroxidase (HRP, Sigma, VI, 10% in 100nl Ringer) into dorsal HIP either at 3 or 21 days post-lesion. Animals were perfused with 2.5% glutaraldehyde 22-24 h after HRP microinjection. The HRP was visualized by the tetramethylbenzidine method.

The total number of neurons in MRN projecting to HIP decreased by 56% 3 days post-CB-lesion as compared to sham injected control (294+48 neurons labeled), but returned to normal levels by 21 days post-lesion. However, at this time the number of labeled neurons in MRN remained significantly reduced (64% lower than sham injected control) in CB-lesioned ADX animals. The ADX control group showed no difference in cell labeling from the sham injected and normal control groups. These results indicate that the CB-lesion induced regrowth in adult rats was suppressed by ADX. Studies are currently in progress to test whether peripheral administration of corticosterone can reverse the suppression of 5-HT homotypic collateral sprouting in the HIP of ADX rats. Supported by NSF-grant BNS-79-06474.

215.38 REACTIVE DEAFFERENTATION IN THE TELEOST MAUTHNER CELL FOLLOWING AXOTOMY. M. R. Wood* and D. S. Faber (SPON: C. M. Smith). Div. Neurobiology; Dept. Physiology; SUNYAB; Buffalo, NY 14214.

Axotomy provides one approach to determining mechanisms regulating membrane properties and trophic interactions. Axotomy of a central vertebrate neuron, the teleost Mauthner (M-) cell, produces a series of morphological and physiological changes characteristic of those described for peripheral and motor neurons (Faber and Zottoli, Brain Res., 223:436-443, 1981). This axon reaction includes chromatolysis and associated ultrastructural changes. Electrophysiological studies revealed the appearance of voltage sensitive membrane in the normally inexcitable M-cell soma. In contrast to most vertebrate neurons, the reaction is not prominent until 40-60 days post axotomy and persists for over 200 days. We now report observations on long-term changes in synaptic inputs to the M-cell following axotomy by complete spinal transection 6 to 9 mm caudal to the soma.

Intracellular recordings from the M-cell soma and lateral dendrite of PSPs evoked by posterior eighth nerve stimulation indicate a reduction in chemically mediated EPSPs generated proximally, while there are no apparent changes in more distal excitatory inputs. The ultrastructure of the M-cell's synaptic bed has been studied from 22 to 207 days post axotomy. Stripping of terminals from the M-cell soma and proximal part of the initial segment has been observed. This deafferentation is characterized by the appearance of thin elements of glia which either: a) completely engulf the terminal, in which case there were no paramembranous densities, or b) partially separate the pre- and postsynaptic membranes. In the latter case synaptic specializations persist in the separated regions, and these partially disconnected terminals still establish synaptic contact with the M-cell. In all such terminals, the synaptic vesicles were predominantly spherical. Deafferentation was not observed on the lateral or inferior dendrites. Axotomy produced a marked increase in extracellular spaces on both the dorsal and ventral soma surfaces. These terminal-free regions of the soma membrane were frequently associated with glia and/or with subsurface cisternae. Finally, terminals exhibited small electron-dense membranous projections which appeared to be either released into the extracellular space around the terminal or engulfed by the M-cell somatic membrane as large coated vesicles.

Partial disconnection may represent an intermediate stage in deafferentation revealed by the slow time course of the M-cell's axon reaction, or it may rather reflect the differential sensitivity to cytoplasmic reorganization of excitatory chemical synapses and other junctions between these cells, such as adherens and gap junctions. (Supported in part by NIH Grant NS 15335)

216.1 PLASTICITY IN THE MOST CAUDAL HINDLIMB DORSAL ROOT GANGLION FOLLOWING GANGLION REMOVAL. M.R. Davis and M. Constantine-Paton, Dept. Biology, Princeton University, Princeton, NJ 08544.

The removal of hindlimb Dorsal Root Ganglia (DRGs) in Rana pipiens results in an increase in cell numbers (hyperplasia) in the remaining DRGs on the ipsilateral side. We have previously observed hyperplasias in positions adjacent to the site of removals and in positions non-adjacent to this site (Davis & Constantine-Paton, 1981, Neurosci.Abst.). The frequent occurrence of non-adjacent hyperplasias in caudalmost ganglion 10 led us to investigate further the nature of plasticity in this ganglion. We report here three unique features of ganglion 10 hyperplasia: bilateral cell numbers increase; it can be produced in animals operated on past metamorphosis; and it is sex-dependent.

Cell counts were performed on ganglion 10 pairs from normal animals and from experimental animals with removals of various hindlimb DRGs. Cell numbers on both the operated and the unoperated side were significantly greater than normal ($p < .05$) yet they were not significantly different from one another. In other hindlimb DRGs the unoperated side showed no increase in cell number and thus served as the control. To determine the extent of hyperplasia in ganglion 10, the bilateral sum of cell numbers was compared to a control value which represented the mean of normal ganglion 10 pairs (N=16).

By this criterion ganglion 10 pairs show a dramatic degree of hyperplasia over a broad age distribution. Tadpole hyperplasias of 57-564% are observed. In animals operated past metamorphosis hyperplasias range from 57-249%. Our studies of the normal changes in neuron cell numbers with developmental age reveal a major decline at mid-larval stages. Mature cell numbers are present from TK XVI onward. Consequently, the production of hyperplasias in post-metamorphic animals is not likely to depend on the massive rescue of cells normally destined to die. Our studies of ^{3}H-thymidine incorporation reveal that neuronal proliferation occurs in normal young post-metamorphic frogs; however, the number of proliferating cells is extremely small.

Dramatic ganglion 10 hyperplasias were found in some animals while in others there were no cell number increases. This led us to examine whether the variability depended on the sex of the frog. We found that ganglion 10 hyperplasias were restricted to males. Of the 17 animals for which the sex was known, all 7 females did not show hyperplasia in 10 whereas they did show hyperplasia in other hindlimb DRGs. No sex differences in cell numbers were observed normally. The basis of this induced sexual dimorphism could depend on either differential connectivity patterns or on systemic factors. But our preliminary analyses reveal no sex differences in peripheral connectivity.

216.2 THE EARLY DEVELOPMENT OF SPINAL PROJECTIONS TO THE BRAINSTEM, THALAMUS AND CEREBELLUM. G.F. Martin. Department of Anatomy and Neuroscience Laboratory, The Ohio State University, College of Medicine, Columbus, Ohio 43210

The organization of spinal projections to the brainstem, thalamus and cerebellum has been described for a number of species including the North American opossum (Hazlett et al., J. Comp. Neurol., 146:95-118, 1971; Brain Res., 33:257-271, 1971), but to our knowledge there have been few reports concerning their development. The opossum is born 12+ days after conception and undergoes a lengthy maturation in an external pouch where it is available for experimental manipulation. We have taken advantage of the opossum's embryology to study the growth of axons from caudal levels of the spinal cord to their definitive bulbar, thalamic and cerebellar targets.

Degeneration techniques were employed on over 75 pouch-young opossums. The spinal cords of anesthetized animals were transected at mid-thoracic levels to elicit degeneration of axons which had grown past the lesion at the age operated. After a variable survival time (6 hours to 7 days depending upon age), the animals were anesthetized and sacrificed by perfusion. The conventional Fink-Heimer technique and the "non-suppressive" Fink-Schneider method were used on frozen sections to impregnate injured axons. The latter technique is particularly good for very immature brains (Leonard, C.M., Brain Res; 53:412-416, 1973).

Our material provides evidence that spinal axons reach the brainstem by at least postnatal day 7 (19 days after conception). At that age they are present within most areas of the bulbar reticular formation innervated by spinal axons in the adult animal as well as within the lateral reticular and inferior olivary nuclei. In contrast, spinal axons were not found in the nucleus gracilis until about postnatal day 16 and the presence of spinal axons in the thalamus could not be documented until about postnatal day 38. A few spinal axons reach the cerebellum by postnatal day 7, but they are not present in the discontinuous patches characteristic of the adult animal (Hazlett et al., Brain Res., 33:257-271, 1971), until approximately 50 days after birth. In summary, our results show that axons from caudal levels of the spinal cord grow into bulbar areas, including precerebellar nuclei, as well as into the cerebellum very early in development. In contrast, direct innervation of the thalamus by spinal fibers as well as indirect innervation via dorsal column pathways appears to occur considerably later.

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216.3 XENOPUS OLFACTORY NEURONS AND THEIR SUPPORTING CELLS ARE DERIVED FROM TWO SEPARATE POPULATIONS. S.L. Klein and P.P.C. Graziadei .Dept. of Biological Sciences, Florida State Univ. Tallahassee, FL 32304.

The origin of the olfactory sensory epithelium of Xenopus laevis has been studied with light microscopy and scanning and transmission electron microscopy. The ectoderm of the Xenopus embryo consists of two layers. The inner layer is called the sensory layer and the outer layer is called the non-nervous ectoderm (NNE). Between stages 23 and 26 (Nieuwkoop-Faber), the presumptive olfactory region consists of two cellular populations: the olfactory placode, a modification of the sensory layer, and the NNE covering it.

After stage 26, the placodal cells begin to move toward the body surface by migrating between the NNE cells. As a placodal cell migrates, it sprouts an apical process which reaches the body surface at approximately stage 28. The apical process contains free and membrane-bound ribosomes, elongated mitochondria, and microtubules oriented parallel to the process. Centrioles and basal bodies are present in the apical cytoplasm and microvilli and cilia project from the process's surface. The structure and ultrastructure of the apical process identify it as the dendrite of the olfactory neuron. As the apical process reaches the body surface, a second process sprouts from the base of the cell. The basal process pierces the basal lamina at stage 29/30 and soon reaches the underlying telencephalon. The basal process is considerably thinner that the apical one, and contains only free ribosomes and mitochondria. Between stage 29/30 and 32^{+}, it terminates as a growth cone. The structural and ultrastructural morphology of the basal process identifies it as the axon of the olfactory neuron. Thus, the placodal cells which migrate superficially differentiate into the olfactory neurons. Many placodal cells do not migrate, but remain at the epithelial base as basal cells.

As the placodal cells migrate to the body surface, the NNE cells, originally confined to the surface layer, elongate and project processes toward the basal lamina. Beginning at stage 27, the apical surface of the NNE cells becomes domed. The apical dome is filled with dense-cored vesicles, and covered with microvilli and a globular material. The supranuclear cytoplasm of this cell contains free and membrane-bound ribosomes, microtubules, and microfilament bundles. These characteristics identify this cell as the supporting cell of the olfactory epithelium. Supported by NSF grant: BNS 8006803

216.4 SENSORY NEURON REPLACEMENT IN THE HAMSTER OLFACTORY SYSTEM WITH AND WITHOUT A TARGET (OLFACTORY BULB). R.M. Costanzo and P.P.C. Graziadei. Dept. Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298 and Dept. Biological Sciences, Florida State University, Tallahassee, FL 32306.

Sensory neurons in the olfactory system are unique in that they are replaced in the adult animal following degeneration of mature sensory receptor cells. These newly formed receptor cells are capable of sending axonal processes to the olfactory bulb, or in its absence, to cells in the forebrain, where they have been reported to make synaptic connections.

We have made a quantitative study of this replacement process in 105 adult hamsters following two separate procedures: (1) Unilateral bulbectomy (target removed) and (2) Unilateral nerve transection (target intact). Ratio measurements (experimental side/control side) were made for epithelial thickness and total cell number (supporting, receptor, and basal cells) and were expressed as percentages for post-op days 1,2,3,4,5,7,10,15,25, 35,60, and 120.

Following bulbectomy (N=63), we observed an immediate degeneration of cells in the olfactory epithelium resulting in a decrease in number to 39% of control by day 4. During days 4-15 there was an increased growth period, resulting in a new population of cells which was maintained at a level 60-70% of control through day 120. Epithelial thickness decreased to 60-75% during the degeneration period; however, it failed to show any recovery during subsequent days 4-120. To study changes in epithelial cell morphology in more detail during the recovery process, we examined an additional group of bulbectomized animals (N=31) using scanning EM.

Following the nerve transection procedure (N=42) we observed similar changes. Cell number decreased to 45% by day 4 and recovered to 55-75% of control. Epithelial thickness decreased to 60-70% with no apparent recovery. Comparison of the bulbectomy and nerve transection data suggests that the presence or absence of the olfactory bulb (target) does not affect the time course of the recovery of epithelial thickness and cell number, nor does it account for the observed failure of the olfactory epithelium to return to control levels.

Supported by NIH Grant NS 16741 to RMC and NSF Grant BNS8006803 to PPCG.

216.5 INFLUENCE OF TARGET TISSUE ON MARKER PROTEIN SYNTHESIS IN OLFACTORY RECEPTOR CELLS. M. I. Chuah* and A. I. Farbman. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

Olfactory marker protein (OMP) is a low molecular weight, acidic, soluble protein found exclusively in the bipolar receptor cells. It is synthesized in the cell body of mature receptor cells and transported both to the dendrite and to the axon up to its termination in the olfactory bulb. This protein has been isolated and a specific antibody raised against it*. In earlier immunohistochemical studies on developing olfactory epithelium, it was shown that OMP is first detectable in the most mature receptor cells of rats on day E18 and of mice on day E14. Furthermore OMP is demonstrable in organ cultures of rat olfactory mucosa explanted on day E15 and grown for 6-7 days in culture. When olfactory mucosa was cultured together with presumptive olfactory bulb there was some suggestion from immunohistochemical experiments that more receptor cells contained OMP. The present study was initiated to determine whether indeed there was more OMP in the cultures containing olfactory bulb. Specimens were taken from E15 rat embryos (day E1 is when the dam is sperm positive) and explanted onto collagen coated Millipore filters supported on stainless steel grids in organ culture dishes. Cultures were grown in Waymouth 752/1 medium supplemented with 0.30mg/ml Ascorbic Acid and 0.10mg/ml Gentamicin, at 35°C in an atmosphere of 5% CO_2. Some cultures contained olfactory mucosa alone and others contained the olfactory mucosa and bulb explanted en bloc. Specimens and media were harvested for the detection of OMP by radioimmunoassay from 4-14 days after explantation. Tissue extracts were obtained by homogenizing the pooled cultures in 100mM Tris and then centrifuging the homogenates. The OMP is quantitatively determined by a solid-phase radioimmunoassay, using ^{3}H-labeled OMP. Results of radioimmunoassay showed that OMP was first detected in cultures of olfactory mucosa and bulb 4 days after explantation and increased up to the sixth day, after which the level of OMP remains constant. In the cultures of olfactory mucosa grown alone, OMP was also detectable on the fourth day; but the maximum amount of OMP was less than that of cultures grown with the bulb at every time point up to 14 days after explantation. At no time was OMP detectable in the culture medium. The results indicate that the olfactory bulb enhances the synthesis of OMP in receptor cells. The data suggest that, as in other developing neuronal systems, the target organ of the developing primary olfactory pathway influences the maturation of the receptor neurons with which it subsequently forms synapses.

*We are grateful to Dr. Frank L. Margolis, Roche Institute of Molecular Biology, Nutley, N.J. for his generous gift of purified OMP and anti-OMP. Supported by USPHS Grant #NS-06181.

216.6 THE USE OF TACTILE AND OLFACTORY CUES IN NEONATAL ORIENTATION. M.Larson* and B.Stein (SPON: K. Corley).Dept. of Physiol., Medical College of Virginia, Richmond, Va. 23219.

A good deal of effort has been directed toward determing the sensory cues employed by neonatal animals in orienting to, and localizing, the nipple. The results of previous studies are contrdictory. Some investigators have claimed that olfactory cues are critical while others suggest that tactile cues are of primary importance in these behaviors. The present studies were initiated because of the possibility that the presence of conflicting views may be due to the analysis of slightly different components of a complex behavioral sequence. Experiments were performed on 10 kittens from 1-56 days of age. In order to test for the involvement of tactile and olfactory cues in neonatal orientation a series of tests on each kitten was administered. The kittens were evaluated as "normals", after the vibrissal pads were anesthetized and after olfaction was disrupted. Three tests per day were run on each kitten for 56 days. Tactile input was impaired by topical application of lidocaine (2% lidocaine jelly) and olfactory input was disrupted by intranasal plugs or amyl acetate. The ability and time involved in localizing and attaching to the nipple of of the anesthetized mother was measured in each of the following conditions: the kitten was a)placed on the cage floor 10cm from the mother, b) on the mother's dorsum, and c)held with its face 2 cm lateral to the nipple with the snout contacting the body. Each test lasted 5 min. Under normal conditions these kittens attached to the nipple in 5-30 secs. regardless of distance from the nipple. With tactile input impaired, kittens did not attach to the nipple on any tests (5 min max time allotment) but had no difficulty orienting and crawling to the mother. In contrast, olfactory disruption never impaired nipple attachment when the kitten was on the mother (test a), or near the nipple (test b), but interfered with the kittens' ability to locate the mother (test c). These data suggest that olfactory cues are employed in locating the mother and tactile cues from the vibrissal pads are employed in locating the nipple. We suggest that previous conflicts may have arisen as a result of analysis of different components of this behavioral sequence of nipple location.

The development of a sensory motor loop involving motor cortex is known to have a protracted developmental time course and could not underly these orientation behaviors (Bruce,I.and Tatton,W., Exp Brain Res., 39:411, 1980). A likely structure mediating these behaviors is the superior colliculus. Tactile-responsive cells are known to exist in the superior colliculus of prenatal animals and the functional integrity of some superior colliculus efferents has already been demonstrated (Stein,B.,Clamann,P., and Goldberg, S., Science, 210:78, 1980). Supported by Grant NS 15912

216.7 THE DEVELOPMENT OF FUNCTIONAL PLASTICITY IN THE FIRST SOMATOSENSORY CORTEX: A (^{14}C)-DEOXYGLUCOSE STUDY IN THE NEONATALLY-DENERVATED RAT. Shyh-Chang Sheu* and Peter J. Hand. Dept. of Animal Bio., Sch. of Vet. Med., Inst. of Neurol. Sci., Univ. of Penna., Phila., PA 19104 and Nat. Yang-Ming Med. College, Taiwan, R.O.C.

Previous (^{14}C)-deoxyglucose (2DG) studies demonstrated that stimulation of vibrissa #3 of row C (C3) in the adult rat produced spindle-shaped metabolic labeling in laminae I-VI of contralateral first somatosensory cortex (SI) (Hand et al., Neurosci. Abst., 1977). Subsequent 2DG studies on rats 3 months after neonatal (postnatal days 2-4) follicle ablation, sparing C3, revealed an enlarged, but diffuse pattern of C3 labeling in contralateral SI. In comparison with the normal C3 barrel in lamina IV, the spared C3 barrel had enlarged by 36% whereas the areal extent of the spared C3 metabolic labeling in lamina IV had increased by 125% over that of normal C3 labeling (Kossut et al., Neurosci. Abst., 1979).

The present investigation examined the effects of survival time upon the development of functional plasticity in SI associated with activation of the spared C3 vibrissa. All vibrissa follicles, excepting C3, of 14 rats were ablated unilaterally on postnatal days 2 and 3. Survival periods ranged from 1-8 weeks (S 1-8). 5-30μCi of 2DG was injected intraperitoneally (S 1-7) or intravenously (S 8) and control and spared C3 vibrissae stimulated as previously described (Hand et al., Neurosci. Abst., 1978). The results based upon serial tangential autoradiograms were as follows: (1) The areal extent of elevated 2DG labeling in SI associated with the spared C3 vibrissa in S 1-8 rats had increased from 25% - 133%, (2) In this period of time bursts of increased areal extents of labeling were observed between 1-3, 4-5, and 7-8 weeks postoperatively, (3) No increased or slightly decreased extents of labeling were observed between 3-4 and 5-7 weeks postoperatively, (4) The pattern of increased 2DG labeling exhibited a directionality in that S 2-4 rats showed a greater areal extent in the same orientation as barrel rows while very short (S 1) or longer survival time rats (S 5-8) showed a more even increase in areal extent of labeling (i.e., along barrel rows and arcs), and (5) Thionin-stained sections revealed that, in comparison to the normal C3 barrel, the spared C3 barrel had enlarged by only 24-36% (S 1-8), exhibited fluctuations in diameter over time, and did not show the progressive increases in size with increasing survival time.

In conclusion, these results indicate that the development of metabolic (functional) reorganization in lamina IV of SI (in response to partial vibrissa denervation in the neonatal rat) is a dynamic and cyclical process exhibiting a progressive enlargement of spared C3 vibrissa labeling over time as well as periods of marked increases (4-5; 7-8 weeks) or slight decreases (3-4; 5-7 weeks) in such labeling. (Supported by grant NS-14935-01 and National Yang-ming Medical College, Taiwan, R.O.C.).

216.8 SOUND LOCALIZATION IS ADJUSTED ACCORDING TO VISUAL SPACE IN OWLS. P. F. Knudsen*, E.I. Knudsen and S.D. Esterly*. (SPON: D. Kuffler) Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Sound localization errors can be induced in young barn owls by chronically distorting their binaural cues. When owls with such sound localization errors have prisms placed over their eyes, they adjust their sound localization according to the visual deviation caused by the prisms.

Sound stimuli were generated by a speaker that could be positioned anywhere around the owl by remote control. Traveling with the speaker, and centered in its cone, was a small LED that provided a light stimulus. The speaker was moved to random locations in front of the owl, and the owl was required to orient its head in response to either a sound or light stimulus. Sound localization error was defined as the difference between the mean orientation to lights and the mean orientation to sounds. Normal owls localize sounds with less than 2.5° error in azimuth and elevation.

By plugging one ear we distorted the owl's binaural localization cues and produced systematic localization errors. We found that the birds adjusted to their ear plugs and after several weeks localized sounds normally. Removing the ear plugs again induced sound localization errors in both azimuth and elevation. For one owl this error was 15.7° right and 16.1° up. The day after the plug was removed, spectacles with prisms that deviated the visual world 12° right were placed on the owl. This owl's sound localization error was measured every other day for 4 weeks. At the end of this time, the owl had adjusted so that it was localizing sounds normally in elevation but had an error of right 13° in azimuth, matching closely the visual error created by the prisms. The prisms were then removed, eliminating the visual error, and after 4 weeks, the owl was localizing sounds normally.

A second owl was also fitted with prisms the day after its ear plug was removed. This owl's initial sound localization error was right 6.4° and up 8.4°. This owl received prisms with a visual deviation of right 6° and up 8° matching closely its auditory error in both dimensions. After 4 weeks, its sound localization error remained at right 5.5° and up 8°. The prisms were then rotated so that the visual deviation was 0° in azimuth and up 9.1° in elevation. Over the next 4 weeks, the owl adjusted its sound localization error to right 0.7° and up 8.9°, thereby matching its visual error. The prisms were then removed and after 4 weeks the owl was localizing sounds normally.

We conclude that the owl uses visual information to correct errors in sound localization.

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216.9 THE DEVELOPMENT OF PHASE-LOCKED RESPONSES IN THE AUDITORY NERVE OF THE KITTEN. Ronald E. Kettner, Jia-Zhen Feng* and John F. Brugge*. Dept. of Neurophysiology and Waisman Center on Mental Retardation and Human Development, University of Wisconsin, Madison, WI 53706.

The phase-locked responses of single auditory nerve fibers to low-frequency tones were studied in kittens during the first month postpartum. Both the degree of phase-locking (vector strength) and the average phase of the locked discharge were computed from period histograms generated by the responses to pairs of three-second tones. In the auditory nerve of the adult cat the degree of phase-locking, recorded at 20-30 dB above threshold, gradually diminishes as stimulus frequency is raised and, for most fibers, is barely recognizeable above some 4-5 kHz. After the first postnatal week the degree of phase-locking, at frequencies below about 600 Hz, is similar to that in the adult. As stimulus frequencies are raised above this, the progressive decrease in phase-locking is substantially greater in the kitten as compared to the adult cat. In the 10-day-old kitten, for example, the degree of phase-locking is far below that in the adult at 1000 Hz and by around 2000 Hz the period histogram shows little sign of phase-locked activity even at tonal frequencies at or near CF. With advancing age phase-locking gradually improves, reaching adult levels toward the end of the third postnatal week. Comparisons between the phase-locked properties of AVCN neurons (Brugge et al., J. Neurophysiol., 41:1557; 1978) and auditory nerve fibers indicate that the development of phase-locking in the AVCN lags that in the auditory nerve by several weeks. Thus, the relatively late development of phase-locking in the AVCN can only be partially accounted for by the development of this pattern of activity in the auditory nerve.

In both the kitten and adult cat the average phase of the locked discharge is a linear function of stimulus frequency. Estimates of cochlear delay times, which include mechanical travel time and neural delays, were obtained by calculating the slope of the phase-versus-frequency curves. Cochlear time delays for a wide range of CFs approached adult values by the end of the first postnatal week. (Supported by NIH Grants HD03353 and NS07026)

216.10 HRP STUDIES OF THE DEVELOPING AUDITORY NUCLEUS IN RANA CATESBEIANA. J. Jacoby* and K. Rubinson. Dept. of Physiology and Biophysics, New York Univ. Med. Ctr., New York, NY 10016.

The dorsal medullary nucleus (DMN) of Rana catesbeiana is the target of all the afferents from the amphibian and basillar papillae and has been shown to be a highly structured processing and relay nucleus for auditory information. The DMN is seen in the tadpole as a discrete group of cells in the dorso-lateral region of the medulla prior to the emergence of the hind limb bud. At this early stage of development, the terrestrial middle ear structures have not yet appeared, although the auditory papillae are formed. We have used anterograde and retrograde HRP techniques in order to assess the morphology of DMN cells and their relation to VIII nerve afferents at these early developmental stages.

The VIII nerve and its branches were cut within the otic capsule of R. catesbeiana tadpoles, and a crystal of HRP embedded in gelfoam was applied to the proximal stump. Retrograde filling of DMN cells was accomplished by the iontophoretic injection of HRP in the vicinity of the contralateral superior olivary nucleus or DMN. Thirty-three micrometer cryostat sections were reacted using the TMB procedure (Mesulam) and counterstained with neutral red.

At all stages beginning with the earliest limb bud stages (Taylor and Kollros I-III) anterograde HRP fills of the entire VIII nerve or of the posterior ramus showed afferent projections to the entire DMN and to the vestibular nuclear zones ventro-medial to it. HRP fills of the amphibian papillary branch alone showed a distribution restricted to the ventral and lateral portions of the DMN at the beginning of hindlimb bud development. At later tadpole stages (VII-X) the projection zone of the amphibian papillary branch extends medially to encompass the lateral half of the nucleus.

Injections of HRP in the contralateral superior olivary nucleus at the earliest limb bud stages (II-III) retrogradely filled DMN cell bodies and dendrites. DMN cells projecting to the contralateral DMN could be definitively demonstrated at midlarval stages (IX-XI). Most of the dendrites of filled DMN cells were oriented towards the center of the DMN and all dendrites were contained within the confines of the nucleus. The axons of these projecting cells exit from the nucleus in a ventro-medial direction, passing lateral to or through the adjacent dorsal island of Kingsbury. These same injections also labelled axons from contralateral nuclei which enter the DMN from its ventro-lateral aspect and terminate proximate to the dendrites of the filled DMN cells.

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216.11 THE POSTNATAL DEVELOPMENT OF THE DORSAL COCHLEAR NUCLEUS IN TREE SHREW. M. T. Ochs* and J. K. Brunso-Bechtold, Dept. of Anatomy, Vanderbilt University, Nashville, TN 37232

As in other mammals, the dorsal cochlear nucleus (DCN) of the mature tree shrew can be divided into three layers oriented parallel to the external surface of the nucleus. The fusiform cell layer (FCL) is bordered externally by the molecular layer (ML) and internally by the polymorphic layer (PL). The FCL contains relatively large fusiform cells oriented perpendicular to the surface of DCN in addition to small and granule cells. Externally, the ML appears rather cell-sparse; it is largely composed of neuropil with some small cells and glia. Internally the PL consists of numerous morphological types of cells scattered throughout the deep part of DCN. Adjacent to the DCN border with ventral cochlear nucleus, as well as on the dorso-lateral surface of DCN, there is a homogenous rim of granule cells.

The laminar characteristics which are so well-defined in the mature animal appear far less obvious in the neonatal tree shrew. First, the FCL is not readily distinguishable from the two other layers. This is not, however, due to an absence of fusiform cells; at birth these cells are present in approximately the same location and have the same orientation as in DCN of the mature animal. The poor definition of the FCL at birth is probably due to changes in the small and granule cell populations in the FCL as well as to a lack of neuropil. Dendritic maturation of the fusiform cells in the hamster has been reported postnatally (Schweitzer, 1981). Another characteristic of DCN at birth is a greatly enlarged granule cell rim. This region occupies a far greater percentage of the nuclear volume at birth than in the adult (47% vs 13%). This change cannot be entirely accounted for by a greater expansion of the non-granule cell portion of cochlear nucleus. Estimates of cell number suggest that at least some of the change appears to be due to a decrease in the number of cells in the granule cell rim as the animal matures. Although cell death cannot be ruled out as a factor, migration of granule cells into the main part of cochlear nucleus is an attractive possibility in light of parallels which have been proposed between cerebellum and cochlear nucleus. This migration of granule cells from the lateral rim, however, must be involved in more than a simple definition of the FCL since that layer is evident by the 4th postnatal day; yet at postnatal day 17 the granule cell rim still forms a far greater percentage of the cochlear nucleus than in the adult (29% vs 13%).
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216.12 RETINAL-IMAGE DEGRADATION PRODUCES OCULAR ENLARGEMENT IN CHICKS. William Hodos and Wayne J. Kuenzel*(SPON: R.J. Dooling). Depts. of Psychology and Poultry Science, Univ. of Maryland, College Park, MD 20742.

Two groups of three-day-old broiler chicks were fitted over the right eye with plastic goggles that degraded the retinal image by producing spherical abberation, reduction of contrast and blurring of edges. In one group of chicks, the goggles were hemispherical and affected the entire visual field. In the second group, the goggles only affected the frontal visual field. In both groups, the left eye was used as a control. A third group of chicks served as an untreated control group.

After three weeks, the chicks were sacrificed. The eyes were removed and hemisected. The hemisected eyes were photographed at 5X magnification and measured in the axial plane and the equatorial plane. Because the avian eye is flattened in the equatorial plane, both sets of measurements are necessary in order to represent changes in its size and shape. The axial length of a laterally-located avian eye is related to distance vision. The equatorial length is related to vision in the frontal field, which is used for near vision.

For each group of chicks, the difference between the right eye and the left eye was compared. The mean difference in the equatorial and axial planes for the untreated control group was 0.03 and 0.01 mm, respectively, which is not significantly different from zero. The mean axial difference for the full-goggle group was 0.33 mm, which is signifiant. The mean difference for this group in the equatorial plane was 1.09 mm, which is very highly significant. For the partial-goggle group, the mean axial difference was 0.07 mm, which is not significant; however, the mean equatorial difference was 0.49 mm, which is highly significant.

A disadvantage of the full goggles is that food particles and condensation collect on the inner surface, which further obscure vision. In some cases, the full goggles were found to interfere with the normal growth of the eyelids. Although the effects of the partial goggles are smaller than those of the full goggles, they do not collect condensation and are easy to clean. Moreover, they do not interfere with the growth of the eyelids nor do they appear to affect distance vision.

These results suggest the increased equatorial length found after retinal-image degradation may serve as an animal model of myopia, which is a condition of the eye in which the distance from the cornea to the retina is too long so that the focus of light rays falls short of the retina.

216.13 DEFECTIVE MIRROR-IMAGE DISCRIMINATION IN PIGEONS: A POSSIBLE ANIMAL MODEL OF DYSLEXIA. Susan R. B. Weiss* and William Hodos. Dept. of Psych., Univ. of Maryland, College Park, MD 20742.

In the course of several experiments, 48 pigeons were tested in a simultaneous-discrimination task using three sets of visual stimuli. Within each set, the stimuli varied only in orientation. One stimulus pair consisted of lateral mirror images and one pair consisted of vertical mirror images. Within the third pair, the stimuli differed in orientation by 90^0 and thus were not mirror images. The majority of the birds tested learned each discrimination rapidly and at approximately equal rates. The median number of sessions required to reach the criterion of 90% correct on all three discriminations for three successive sessions was 14.

Approximately 20% of the subjects acquired the discriminations more slowly than normal. The performance of these subjects remained at chance levels for a longer period of time and they failed to reach criterion by 30 or more sessions. A consistent pattern emerged in the data of these subjects; i.e., in all cases except one, performance was poorest on the lateral mirror-image stimuli. The one exception was poorest on the vertical mirror-image pair. Although some of these birds did reach 90% correct on the non-mirror-image or vertical mirror-image stimuli, none reached 90% correct on the lateral mirror-image stimulus pair.

The first of the poorly-performing pigeons to be encountered were discarded from the experiments. Later cases of this sort were transferred to other experiments, which used psychophysical techniques to measure visual acuity, intensity-difference threshold and line-orientation-difference threshold. In each case, their sensitivity was within the normal range.

In humans, dyslexia is a deficiency in reading ability observed in children. The disorder is often characterized by a difficulty with letters that are lateral mirror images such as b and d. Dyslexic children typically are unimpaired in other aspects of intellectual ability or visual sensitivity. The similarity between the reading difficulties of dyslexic children and the mirror-image discrimination difficulties of this sub-population of pigeons suggests that these pigeons might serve as a useful animal model of the human disorder.

216.14 EFFECTS OF BICUCULLINE-INDUCED EPILEPTIFORM ACTIVITY ON THE DEVELOPMENT OF RECEPTIVE FIELD PROPERTIES IN THE LATERAL GENICULATE NUCLEUS AND VISUAL CORTEX OF YOUNG RABBITS. Louis H. Ostrach, John W. Crabtree*, Bruce G. Campbell*, & Kao L. Chow. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

Previous studies have shown that normal development of receptive field properties in cells of the lateral geniculate nucleus (LGN; Baumbach and Chow, Brain Res. 209:61-76, 1981) and of the visual cortex (Crabtree et al., Dev. Brain Res.1:269-281, 1981) is disrupted by chronic, penicillin-induced, epileptiform activity in the visual cortex of the rabbit. It is important to know whether the developmental disruption found in both structures resulted from some manifestation of the cortical paroxysmal discharges or from some toxic peculiarity of penicillin itself. To test the latter possibility the above studies were replicated using a different neuroleptic drug, bicuculline.

In 7-8 day rabbit pups a stainless steel cannula was implanted over each visual cortex so that the cannula rested on the dura overlying the monocular visual area. Beginning on the following day, applications of bicuculline (3.3mM in 0.9% saline) to the dural surface through one cannula were made twice daily for 11-17 and 17-23 consecutive days for studies of the LGN and visual cortex respectively. Following drug applications interictal spiking was observed within 10 minutes on the EEG monitor and lasted for 4-6 hours. Concurrent saline applications through the other cannula provided a control procedure for surgical and drug administration effects. Interictal spiking was not seen in the control cortex. Drug applications were discontinued 24 hours before electrophysiological recording. Responses of single units in the LGN were recorded on postnatal day 20-25; single unit responses in visual cortex were recorded on day 25-31.

In the LGN ipsilateral to the bicuculline-treated cortex the percent distribution of cells with various receptive field types did not differ significantly from that distribution obtained for normal LGN. However, the cell percent distribution of receptive field types in the ipsilateral visual cortex was significantly different from the control cortex distribution. There was a significant increase in the proportion of unresponsive and indefinite type cells and a concommitant decrease in the proportion of cells having linearly oriented receptive fields. Relative to control cortex values, the developmental abnormalities found in bicuculline-treated visual cortex are virtually the same as those found in penicillin-treated visual cortex. These results lend further support to our previous conclusion that, in the rabbit, early alteration of intrinsic cortical activity prevents the normal development of the full complement of cells with oriented type receptive fields. (Supported by NS12151, EY05382 & EY00691).

216.15 DEVELOPMENTAL CHANGES IN TASTE RESPONSES FROM RAT SOLITARY NUCLEUS. D. L. Hill Center for Human Growth & Devel. Univ. of Michigan, Ann Arbor, MI 48109.

Developmental changes in peripheral taste nerve responses have been observed in the rat. For example, response frequencies to NaCl and LiCl increase substantially from 20 days to 35 days and frequencies to citric acid decrease. To determine whether similar changes occur in central nervous system responses, electophysiological recordings were made from chemosensitive neurons in the nucleus of the solitary tract (NST) in developing rats. Twenty-nine single units were studied in rats aged 14-20 days, 26 units in rats aged 25-35 days, 26 units in rats aged 50-60 days and 31 units in adult rats. In addition, multiunit taste responses were recorded from the NST in 6 rats aged 5-7 days. Chemical stimuli applied to the anterior tongue were 0.1M and 0.5M NH_4Cl, NaCl, LiCl and KCl, 0.1M citric acid, 0.01N HCl, 1.0M sucrose, 0.1M Na-saccharin and 0.01M quinine HCl. Neural activity was measured for the first 5 sec after stimulation of the tongue; a comparable period of prestimulus spontaneous activity was subtracted to yield response frequencies.

NST neurons in rats aged 5-7 days are characterized by a general absence of responses to 0.1M salts and to 0.5M NaCl and LiCl. However, 0.5M NH_4Cl and 0.1M citric acid repeatedly elicited responses in all recordings. In contrast, all single units in older rats responded to each monochloride salt and citric acid. Therefore, changes occurred in central taste responses between 7 and 14 days. Further changes in central taste responses were apparent later in development. Response frequencies to NaCl, LiCl, KCl, sucrose and saccharin in rats aged 14-20 days and 25-35 days were significantly lower than those in rats aged 50-60 days and in adults ($p<0.01$). Responses to NH_4Cl, citric acid, HCl, and quinine did not change after 14 days of age, however ($p>0.20$).

These results suggest that chemosensitive responses in the NST develop first and mature earlier for relatively aversive stimuli (e.g., NH_4Cl, citric acid, quinine HCl). Maturation of responses to nutritive stimuli (e.g., NaCl and sugars) occurs more gradually, since response frequencies to these stimuli are still increasing after 35 days of age.
(Supported by NIH Grant NS17404)

216.16 COMPARISON OF DEVELOPMENTAL CHANGES IN SALT TASTE RESPONSES FROM ANTERIOR AND POSTERIOR TONGUE. C.M.Mistretta and R.M.Bradley Dept. Oral Biol.,S. Dent.,Univ. of Michigan, Ann Arbor,MI 48109.

Salt taste responses recorded from the chorda tympani nerve during chemical stimulation of the anterior tongue change developmentally in sheep. To learn whether developmental changes are also observed when taste buds in circumvallate papillae on the posterior tongue are stimulated, we have recorded from the glossopharyngeal nerve. Multifiber responses were recorded during stimulation with 0.5M NH_4Cl, KCl, NaCl and LiCl in five age groups of animals: 10 fetuses at about 110 days of gestation (term=147 days); 9 fetuses at about 130 days; 10 perinatal animals aged 142 days of gestation to 7 days postnatal; 10 lambs aged 30-40 days postnatal; 6 adult ewes aged 2-4 years. To compare response magnitudes among different animals in various age groups, we calculated a ratio of every response relative to a standard, frequently applied stimulus, 0.5M NH_4Cl. Mean ratios for each salt were then compared among age groups.

Developmental changes, at statistically significant levels ($p<0.05$), were observed in salt taste responses from the posterior tongue, but the changes are different than those from the anterior tongue. For example, for the anterior tongue there is a marked increase in the relative stimulating effectiveness of NaCl and LiCl throughout pre- and post-natal development. In contrast, glossopharyngeal nerve responses to posterior tongue stimulation with NaCl and LiCl increase only slightly, relative to NH_4Cl. For the anterior tongue, responses to KCl decrease relative to NH_4Cl but the change is comparatively small. KCl responses from the posterior tongue also decrease in magnitude, but the change is much greater than for the anterior tongue. The extent of the changes from the youngest to the oldest age group are summarized:

RESPONSE RATIOS

	Chorda				Glossopharyngeal			
	NH_4	K	Na	Li	NH_4	K	Na	Li
110 day fetus	1.0	0.8	0.2	0.2	1.0	1.8	0.2	0.2
adult	1.0	0.6	1.0	1.0	1.0	0.7	0.4	0.5

Thus, for taste buds in very different anatomical arrangements and locations on the tongue, developmental changes occur in salt taste responses. But the changes are dissimilar for the anterior and posterior tongue. These results predict that the various membrane components interacting with salts are present in different proportions in fungiform and circumvallate taste buds at different times in development. Furthermore, for either anterior or posterior tongue, different membrane components must be responding to NH_4Cl or KCl, compared to NaCl and LiCl. (Supported by N.S.F. Grant BNS80-15737 and Research Career Development Award, N.I.H., N.I.D.R., DE-00066 to C.M.M.)

216.17 SALT TASTE RESPONSES IN RATS DEPLETED OF NaCl DURING EARLY DEVELOPMENT. R.M.Bradley, Dept. Oral Biol., Sch. Dent. D.L. Hill and C.M.Mistretta. Center for Human Growth & Devel. and Dept. Oral Biol., U. Mich., Ann Arbor, MI 48109.

Recently we demonstrated that there are developmental changes in taste responses to NaCl, LiCl, NH_4Cl and KCl recorded from the rat chorda tympani nerve. We have now attempted to manipulate these changing responses by depleting NaCl in developing rats. Eleven pregnant rats were fed a sodium-free diet from 3 days of gestation until 12 days after birth; thereafter, they received the same diet with 1% NaCl added. Only 3 rats delivered live young to yield a total of 13 sodium-depleted pups. Six pregnant control rats were fed the 1% NaCl diet and were treated in the same manner as experimental animals. Thirty-four control pups were delivered.

All pups were weaned at 30 days, maintained on the 1% NaCl diet, and at 58 days were preference-tested with water versus 0.001 to 0.5M NaCl (6 concentrations, tested on alternate days). Since depleted rats were smaller than controls, all ingestion values were corrected for body weight. Salt-depleted rats exhibited a decreased preference score for NaCl across concentrations ($p<0.05$); however, they drank more NaCl ($p<0.05$) and more water ($p<0.0001$) than controls. To account for the decreased preference relative to controls, it is obvious that water intake in depleted rats was proportionately greater than NaCl consumption.

After 12 days of preference-testing, neurophysiological responses were recorded from the whole chorda tympani nerve in 13 depleted rats and 15 control rats. Stimuli were 0.1M NaCl, LiCl, NH_4Cl, KCl and concentration series of 0.01 to 0.5M NaCl, LiCl, NH_4Cl and KCl. To compare responses to 0.1M salts among animals, integrated, steady-state responses were measured and ratios were calculated for each salt relative to a 'standard' salt (NaCl, NH_4Cl or KCl). Whether responses were expressed relative to NaCl, NH_4Cl or KCl as a standard, no differences were found between sodium-depleted and sodium-fed groups for any of the salt responses ($p>0.10$). Furthermore, there were no differences between groups for NaCl, NH_4Cl or KCl response-concentration functions ($p>0.10$).

Therefore when rats undergo NaCl depletion during early development, peripheral taste nerve responses to various salt stimuli recorded at two to three months of age are not altered. However, salt taste responses may have been altered at an earlier age (near the time of depletion) and may have then returned to normal values. This remains to be determined. Also, more data are needed to establish whether the sex of depleted animals is related to salt responses. (Supported by NIH Grant NS17404 and NSF Grant BNS80-15737).

216.19 ALTERATIONS IN FUNCTIONAL PROPERTIES OF TRIGEMINAL BRAINSTEM NEURONES SUBSEQUENT TO TOOTH PULP DEAFFERENTATION. J.W. Hu*, J.O. Dostrovsky, B.J. Sessle and M. Sirisko*. Faculties of Dentistry and Medicine (J.O.D.), University of Toronto, Toronto, Canada M5G 1G6.

We previously reported (Ball et al., Soc. Neurosci. 1979) that partial tooth pulp deafferentation of mandibular teeth may be associated with functional alterations in trigeminal (V) brainstem neurones in adult cats. We have now extended this preliminary study to examine these effects more quantitatively, to obtain information on their time course, and to determine the effects of maxillary as well as mandibular pulp deafferentation. Single neurone recordings were made in a series of systematic microelectrode penetrations from the subnucleus oralis of the V spinal tract nucleus of chloralose-anaesthetized or unanaesthetized (decerebrate) cats at a single post-operative time that varied from 3-140 days after removal of the coronal pulp of the right maxillary or mandibular canine, premolar and molar teeth. To obviate the possibility of experimenter bias, some of the operated and unoperated (control) cats were prepared for investigation so that the pulp condition (intact or not) was not made known to the investigators. Natural and electrical stimuli were applied to various superficial orofacial sites; in addition, four of the remaining left and right tooth pulps of operated cats and six pulps in control cats were electrically stimulated.

A total of about 2,000 neurones in the ipsilateral subnucleus oralis was studied in 50 cats. Deafferentation of either the mandibular or maxillary tooth pulps was found to result in a 50% reduction in the proportion of neurones having receptive fields localized within the V division containing the deafferented pulps. This was associated with a disruption of the normal "inverted-head" pattern of somatotopic organization of the subnucleus. In addition, the incidence of spontaneously active neurones and neurones with abnormal responses (e.g. discontinuous receptive field, habituation) to orofacial stimuli increased from 5% and 1% respectively in control animals to 35-40% and 10-12% after deafferentation. This data relates to changes 7-15 days after pulp deafferentation, but alterations were apparent as long as 140 days after deafferentation. These findings indicate that partial deafferentation of a sensory input that is predominantly if not exclusively related to nociception can lead to marked functional changes in brainstem neurones of adult animals.

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216.18 TRANSIENT EXPRESSION OF CATECHOLAMINERGIC TRAITS IN CRANIAL NERVE GANGLIA OF THE EMBRYONIC RAT. G.M. Jonakait, K.A. Markey, M. Goldstein and I.B. Black. Division of Developmental Neurology Cornell Univ. Med. Coll., 515 E. 71st St. New York, New York 10021 & Dept. of Psychiatry, New York University Medical Center, New York, New York 10016.

In previous studies we have described and characterized a population of cells in embryonic rat gut which transiently expresses several noradrenergic phenotypic traits (Cochard, et al, 1978; Jonakait, et al., 1979, 1980, 1981). We now describe neurectodermal embryonic cells in several cranial nerve ganglia which also transiently express catecholaminergic traits during development.

A few cells expressing immunoreactivity to tyrosine hydroxylase (T-OH), the rate-limiting enzyme in catecholamine biosynthesis, are initially detected in the trigeminal ganglion anlage on embryonic day 11 (E11.0; 24 somites). This precedes the expression of T-OH in sympathetic ganglia and gut. Cells in the trigeminal ganglion anlage at this stage are bipolar, brightly fluorescent, and extend processes into the primitive brain stem. Isolated T-OH-positive cells are also evident medial and caudal to the developing eye cup.

By E11.5 (27-28 somites) a few T-OH-positive cells have appeared in sympathetic ganglia and gut (Cochard, et al., 1978; Teitelman, et al., 1978). In the semilunar ganglion a cluster of T-OH cells is visible at this age. Moreover at E11.5, cells expressing T-OH are apparent in primordia of sensory ganglia serving the glossopharyngeal (IX) and vagal (X) cranial nerves.

By E12 (35-36 somites) only a few T-OH cells in the trigeminal ganglion are detectable, and these occur in the ophthalmic and maxillary divisions. No T-OH-positive cells are observed in the anlage of the vestibulofacial nucleus at this stage. However, unipolar cells expressing diffuse immunoreactivity to T-OH can be seen throughout the more caudal nodose and petrosal ganglia. Moreover, isolated bipolar cells expressing T-OH are visible in rostral dorsal root ganglia at this stage.

Immunoreactive T-OH is undetectable in all of these ganglia by E13 (46-48 somites).

In sum, T-OH transiently appears in cells of cranial ganglia in a rostro-caudal manner. Cells of the early-forming, rostral V^{th} ganglion express T-OH before more caudal ganglia, and before cells of the periphery. Although the role of T-OH in these developing structures and the fate of these cells is unknown, transient embryonic expression of catecholaminergic characters may be a generalized phenomenon. (This research has been supported by grants NS17814, NS10259 and HD12108 from the NIH and from The Council for Tobacco Research-U.S.A., Inc).

217.1 CHANGES IN DENDRITIC ORGANIZATION OF SPINOCERVICAL TRACT NEURONS FOLLOWING PARTIAL CHRONIC DEAFFERENTATION. M.J. Sedivec, J.J. Capowski*, J. Ovelmen-Levitt* and L.M. Mendell, Dept. of Neurobiol. & Beh. SUNY at Stony Brook, Stony Brook, N.Y. 11794 and Dept. Physiol., Univ. of No. Carolina, Chapel Hill, N.C. 27514 and Dept. Physiol., Duke University, Durham, N.C. 27710.

We have filled antidromically identified spinocervical (SCT) cells in the cat L6 segment with HRP using intracellular iontophoresis (Sigma Type VI, 10% solution, 5-20nA, 100Hz for 2-10min). Two preparations were used: intact spinal cord and spared root preparation (L5, L6, S1, S2 cut 60-100 days previously, L7 spared). We examined the effects of partial deafferentation on the structure of an identified population of neurons receiving monosynaptic input from the periphery (Hongo et al, J. Physiol. 199:569, 1968). Initially, we prepared camera lucida reconstructions in which the entire neuron was projected on the sagittal plane (the plane of section). These SCT neurons were oriented in the rostrocaudal axis in agreement with previous work (Brown et al, J. Physiol. 270:747, 1977). Using a computer data tablet we measured the total length of each dendritic tree. Individual neurons (N= 13) in intact preparations had total lengths ranging from 13,455μ to 32,838μ with a mean of 26,255μ. Similar values were observed for 9 SCT neurons in the chronically deafferented L6 segment (Range: 14,532μ to 36,179μ; Mean 26,509μ). Physiologically this population of SCT neurons differed from those in intact preparations by having a greater probability of responding to nociceptive inputs (90% to 30%, respectively). A subset of 8 cells in intact and 5 cells in chronically deafferented (spared root) preparations was subjected to detailed analysis using a computerized neuron reconstruction system (Capowski & Sedivec, Computers & Biomed. Res. 14:518, 1981). This method generally yielded total dendritic lengths about 25% greater than camera lucida reconstructions presumably because total path length rather than the sagittal projection was measured. The most consistent change was a reduction in spines on deafferented neurons. A small but consistent increase in mean diameter of dendritic processes was observed in the proximal portion of the dendritic tree in chronically deafferented cells, resulting in a mean surface area increase of 19% and a mean volume increase of 27%. A selective loss of distal processes dorsal to the cell body was noted. This may help to explain previous findings of distal dendritic atrophy under these conditions (Brown et al, Exp. Neurol. 64:453, 1979) since their conclusions were based on transverse sections through the soma. We conclude that following chronic dorsal rhizotomy SCT neurons undergo reorganization of their dendrites with a loss of spines and net increase in dendritic surface and volume. This results primarily from an increase in diameter rather than length. (Supported by NIH grants NS14899 and NS16996).

217.2 ALTERED DISTRIBUTION OF TRIGEMINAL MANDIBULAR GANGLION CELLS AND THEIR BRAINSTEM PROJECTIONS IN HAMSTERS SUBJECTED TO NEONATAL INFRAORBITAL DEAFFERENTATION. J. Fiore*, M. Math*, R. W. Rhoades and M. F. Jacquin. (SPON: H. P. Zeigler). Dept. of Anatomy, N.J. School of Osteopathic Med. and Rutgers Med. School, P.O. Box 55, Piscataway, N.J. 08854.

Hamsters were subjected to section of the left infraorbital nerve and cauterization of the mystacial vibrissae on the same side within 12 hr of birth. Sixty-70 days later, HRP crystals were applied to the proximal stumps of both mandibular sensory nerve trunks which were sectioned just distal to the foramen ovale. After 3-4 days, both V ganglia and the brainstem were processed for HRP reaction product. Substantial differences were noted between the distributions of labelled neurons in the two ganglia and also between the transganglionic transport to the two sides of the brainstem. In the left V ganglion labelled peripheral fibers occupied an abnormally wide region and labelled mandibular cell bodies were also much more widespread than on the intact side. Such cells were visible at all dorsoventral levels in both the posterolateral and anteromedial portions of the ganglion. There was also a concomitant widening of the portion of the sensory root encompassed by labelled mandibular fibers.

In the brainstem, labelled mandibular axons on the lesioned side occupied a greater portion of the V spinal tract at all rostrocaudal levels and also innervated abnormally extensive territories in all trigeminal subnuclei.

The fact that mandibular fibers innervated regions normally occupied by infraorbital axons was verified in additional neonatally lesioned hamsters in which both the undamaged and regenerated infraorbital nerves were sectioned and exposed to HRP. While there was considerable regeneration on the lesioned side, the number and portion of the ganglion occupied by infraorbital cell bodies was substantially reduced. In the brainstem, labelled infraorbital axons innervated all trigeminal subnuclei but the regions encompassed by such axons were considerably reduced in subnucleus principalis, oralis and interpolaris. In subnucleus caudalis, differences between the infraorbital innervation of the two sides were, at best, slight. Suprisingly, in view of this finding, ectopic mandibular axons were equally prominent in this subnucleus.

Experiments now in progress will determine whether or not the greater area encompassed by the mandibular projection to the brainstem in the lesioned animals represents sprouting and/or the failure to retract of a normally transient "exuberant" projection.

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217.3 MAINTENANCE OF MOTOR ENDPLATE STRUCTURE AND ACETYLCHOLINE RECEPTOR NUMBER BY INNERVATION: DENERVATION VS. DISUSE. S. S. Labovitz*, N. Robbins and M. A. Fahim. Dept. Anat., Case West. Res. Schl. Med., Cleveland, OH 44106.

The neuromuscular post-synaptic apparatus undergoes remarkable changes after short periods of denervation or disuse, e.g. an increase in junctional length occurs after only 7 days of tetrodotoxin (TTX)-induced inactivity (Pestronk & Drachman, Science, 199: 1223, 1978). Therefore, we have studied the structure and acetylcholine receptor (AChR) content of TTX-disused and denervated end-plates.

Sprague-Dawley rats (90-110 g. wt.) were subjected to either unilateral sciatic denervation or chronic nerve block by TTX delivered locally from a minipump implant. Contralateral sham operations or minipumps were also used. 4 to 7 days later, the extensor digitorum longus (EDL) and soleus (SOL) muscles were removed and after labelling with ^{125}I-bungarotoxin, fixation, cholinesterase staining, and dissociation, endplate regions (35-100 μa long) were microdissected from dissociated single fibers for counting of radioactivity and measurement of endplate area (Robbins, et. al., Proc. Roy. Soc., 209:555, 1980). Other muscle samples were prepared for scanning electron microscopy (Desaki & Uehara, J. Neurocyt., 10:101, 1981).

Certain findings were common to both denervation and (TTX) disuse: muscle fiber diameter was reduced to the same degree (Table) and number of junctional AChR per synapse was unchanged from controls (e.g. 5.9 x 10^6 binding sites per synapse innervated SOL, 6.1 x 10^6 sites denervated). However, endplate area of denervated muscle declined in proportion to fiber diameter whereas in disused muscles, with similar fiber atrophy, endplate area was preserved (Table) although in an elongate shape (Table). S.E.M. of denervated SOL showed flattened primary clefts, which were not present in disused muscle. Further analysis of S.E.M. is in progress.

		Fiber Diameter	EP Area	EP Width	EP Length
EDL	Den./Inv.	.73*	.76*	.80*	.94
	TTX/Inv.	.85*	.95	.88*	1.06
SOL	Den./Inv.	.61*	.57*	.74*	.80*
	TTX/Inv.	.65*	1.03	.82*	1.2*

*ratio significantly different from 1.0 ($p<.05$)

Data are from 44 to 170 fibers in 4 to 6 animals.

In disused muscle, presence of an intact axon may preserve the overall post-synaptic specialized area (perhaps by affecting the extracellular matrix), but not the specific dimensions, which are distorted by fiber atrophy. In contrast, when the nerve terminal is removed by denervation, the junction passively follows the diminution in fiber surface area. Junctional AChR number is preserved after both disuse or denervation, despite topologic changes of the endplate. Supported by NIH Grant AG 00795.

217.4 CONTRALATERAL DENDRITIC EXPANSION INDUCED BY AXOTOMY OF FROG SPINAL MOTONEURONS: A Golgi-Computer Reconstruction Study. B. M. Rosenthal, W.L.R. Cruce, Neurobiology Program NEOUCOM, Rootstown, OH 44272.

In a study of the anatomy of the dorsolateral dendritic tree of frog lumbar motoneurons, (Rosenthal, Cruce, and Carlsen, 81) we found that changes occurred in the radial distance to the peak of dendritic branching at 35 days following axotomy. Using Sholl analysis for post-axotomy time periods of 21, 35, and 90 days, our data now suggest an expansion of the dendrites of neurons contralateral to axotomized motoneurons rather than retraction of axotomized motoneuron dendrites. At 21 days the ipsilateral (240 ± 58.8μ) and contralateral (234.2 ± 34.5μ) distances to peak were not different. Likewise at 90 days the ipsilateral (224.2 ± 21.7μ) and contralateral (270 ± 123.5μ) values were not different. Furthermore all these values were not different from the ipsilateral distance to peak (221.7 ± 45.5μ) at 35 days. However at 35 days the contralateral distance to peak (400 ± 68.5μ) was significantly greater than all other values. There were no significant differences in the number of intersections at the peak of dendritic branching, length, surface area, or volume of dendritic trees. These findings do not support the notion that differences in dendritic branching are artifacts of incomplete Golgi impregnation. In an attempt to localize the changes in branching, we subtracted the distance to peak branching from distance to terminal branching ($d_{term} - d_{peak}$) for each cell. There were no differences in this parameter between axotomized and contralateral trees suggesting that the observed effect of increase in distance to the peak of dendritic branching can be explained by a localized effect on the primary dendrites of motoneurons contralateral to axotomized motoneurons. Significance of all results was determined by ANOVA test at .05 level. Supported in part by a BRS Support Grant 2 S07 RR05 806 to W.L.R.C. and B.M.R. and Sigma Xi Grant-in-Aid of Research to B.M.R.

217.5 CA-UPTAKE AND ATPASE ACTIVITY OF SARCOPLASMIC RETICULUM FROM EXTENSOR AND SOLEUS MUSCLE IN SPINAL CORD TRANSECTED RATS. R.J. Boegman, B. Scarth* and K.K. Wan*. Dept. of Pharmacology, Queen's University, Kingston, Ont. Canada

The spinal cord of adult male rats was transected at the mid thoracic level. Ca-uptake and ATPase activity of fragmented sarcoplasmic reticulum (FSR) isolated nine days after the operation from inactive soleus and extensor muscle was assayed. The operation produced a marked increase in the ability of the FSR from the soleus to accumulate Ca in the presence of oxalate. Control soleus FSR accumulated 85.6 nmole Ca/mg prot/min while after spinal cord transection the valve increased to 248.2 nmole Ca/mg prot/min. In contrast to the soleus the operation had little effect on the Ca uptake by the extensor (control 911.1 and experimental 875.8 nmole Ca/mg prot/min.) In the presence of Pi soleus FSR from the experimental animals accumulated more Ca then did the control soleus (28.6 nmole Ca/mg prot/min and 12.0 nmole Ca/mg prot/min). The basal ATPase activity of the FSR increased by approximately 58% in the extensor following spinal cord transection while in the soleus there was a 7% increase. Total ATPase increased by 45% in the extensor and 27% in the soleus following spinal cord transection. Ca uptake in the presence of oxalate by FSR from the extensor and soleus nine days after denervation does not change, however, in the presence of Pi there is a decrease in Ca uptake by extensor FSR. Our data suggests that muscle inactivity produced by spinal cord transection has a different effect on Ca mobilization by FSR than does muscle inactivity produced by denervation.

Supported by the Canadian Muscular Dystrophy Association.

217.6 EFFECT OF SENSORY DENERVATION ON THE POSTNATAL DEVELOPMENT OF MERKEL CELLS WITHIN THE RAT TOUCH DOME. C.A. Nurse*, L. Macintyre* and J. Diamond. Dept. of Neurosciences McMaster University, Hamilton, Ontario L8N 3Z5.

Touch domes (Haarscheiben) in mammalian skin are discrete regions of elevated epidermis containing a basal plate of many Merkel cells, the presumed targets for the SA I mechanosensory axons. Conflicting views persist as to whether the Merkel cells survive denervation in the mammal (e.g. English: J. Comp. Neurol. 172: 137; Hartschuh and Weihe: Neurosci. Lett. 5: 327); discrepancies would not be surprising in view of sampling difficulties intrinsic to the use of electron microscopy for Merkel cell identification. We have re-investigated this question at the developing rat touch dome using a greatly improved sampling technique in which the Merkel cells are made visible in whole mounts by the fluorescent dye, quinacrine (Nurse et al., 1981; Neurosci. Abstr. 135.16); recently we have confirmed at the ultrastructural level the Merkel cell identity of the analogous quinacrine fluorescent cell (QFC) in rat vibrissae (in preparation). For the touch dome studies selected dorsal and lateral cutaneous nerves (T10-L3) were removed in 1-8 week old rats. At various times thereafter the region of denervation was established electrophysiologically and on occasion behaviorally, in animals that received prior dye injection. The average number of QFC/dome ($\bar{X}$) was obtained from samples of 20-50 adjacent domes in the denervated region ($\bar{X}_D$) and compared to that from a corresponding innervated region ($\bar{X}_I$) on the contralateral side (in a few unoperated controls the distribution of QFC/dome was similar from side to side). In normally innervated domes, the average number of QFC/dome increases rapidly (2-3 fold) during the first 3 weeks and reaches a plateau of about 90 QFC/dome by 8 weeks after birth. In 5 animals, domes denervated at 1 week and examined 5-6 weeks later, had about 75% fewer QFC than innervated controls, ($\bar{X}_D$=19.7 $\pm$ 8.1 (SD), n=140 domes; $\bar{X}_I$=83.2 $\pm$ 27.5, n=146 domes). This loss in QFC due to denervation at 1 week did not appear to be simply a curtailment of their normal developmental increase since at age 1 week $\bar{X}_I$=57.1 $\pm$ 28.3 SD (n=265 domes). From studies presently completed it appears that the later the denervation is performed the smaller is the loss in QFC/dome. The loss of QFC due to denervation in these young rats was apparent by 18 days and persisted for at least 100 days. Experiments in progress will test whether this loss in QFC is due to (i) disappearance of Merkel cells (ii) a loss of the ability of some of them to take up or store the dye and (iii) changes in local circulation.

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217.7 HIPPOCAMPAL EXCITABILITY AND RESPONSES TO ACETYLCHOLINE AFTER MEDIAL SEPTAL LESIONS. N. Ropert, K. Krnjević, J.L. Bossu* and J. Davis, Depts. of Anaesthesia Research & Physiology, McGill University, Montréal, Québec and Dept. of Medicine (Neurology) and Pharmacology, Duke University, N.C.

Responses to fimbrial or perforant path stimulation were examined in the hippocampus of rats under urethane, 3-5 weeks after bilateral lesions of the medial septum or after sham operations (The experiments were performed "blind"). The major differences observed in lesioned animals include the following. In the CA1 region, there was an increased excitability of pyramidal cells, manifested by a sharply reduced threshold for evoking population spikes, an increased amplitude of such spikes, and a greater tendency for them to occur in bursts. In area CA3, the increased excitability resulted in striking spontaneous discharges of population spikes, as well as augmented responses to fimbrial or perforant path stimuli. By contrast, there were opposite changes in the dentate gyrus: the threshold for population spikes evoked by stimulating the angular bundle tended to be raised, and the maximum amplitude of spikes was reduced. These results are the reverse of what might be expected in view of the normally predominant facilitation of CA1 and CA3 population spikes by medial septal stimulation or by ACh (Krnjević, K. & Ropert, N., 1981, Can. J. Physiol. Pharmacol., 59:911), as well as the mainly depressant effect of ACh in the dentate gyrus. Possible explanations for these apparently paradoxical findings include: 1. A strong facilitatory action of sympathetic fibres that grow into the hippocampus after medial septal lesions (Crutcher & Davis, Trends in Neuroscience, March 1981, p. 70); the results of systematic stimulations of superior cervical ganglia (Krnjević et al., this meeting) do not favour this possibility. 2. Greatly enhanced efficacy of remaining cholinergic fibres owing to major loss of cholinesterase (confirmed in subsequent histological preparations); a potentiation of the action of ACh was indeed observed in all pyramidal areas. 3. Removal by the septal lesion of a tonic non-cholinergic (perhaps aminergic) inhibitory input to the hippocampus. 4. Loss of a trophic action that normally lowers the excitability of hippocampal neurons. The third and fourth possibilities seem more likely than the first two.

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217.8 TIME COURSE OF CHANGES IN DENDRITIC MORPHOLOGY IN N. LAMINARIS FOLLOWING DEAFFERENTATION. J.S. Deitch* and E.W Rubel. Dept. of Otolaryngology, and The Neuroscience Program, University of Virginia Medical Center, Charlottesville, Virginia, 22908.

N. laminaris (NL), a third-order auditory nucleus in the avian brainstem, is a monolayer sheet of cells whose dendrites are spatially segregated into dorsal and ventral domains. The only known input to the dorsal dendrites arises from the ipsilateral N. magnocellularis (NM), while the ventral dendrites receive matching afferents from the contralateral NM via the crossed dorsal cochlear tract. This symmetrical binaural input is complemented by symmetrical dorsal and ventral dendritic sizes of the cells in NL (r = .71) in normal chickens. Transections of the crossed tract deafferents the ventral dendrites, whereas the input to the dorsal dendrites remains intact. We have used this manipulation to examine the time course and localization of changes in dendritic fields upon removal of this afferent input. Chickens were operated upon at ten days old and allowed various survival times. Normal and sham-operated chickens served as controls. Brain stem cells were impregnated by a Golgi-Kopsch modification and the dendritic length measured from camera lucida drawings. In control chickens the mean percent difference in length between the dorsal and ventral dendrites of an NL cell is 4.6%. Following deafferentation, there were no changes in the length of the dorsal dendrites. In contrast, the ventral dendrites underwent a rapid change in length; the mean percent difference between dorsal and ventral dendritic length was 28% within 12 hours and 29% at 24 hours. The difference was 34%, 42%, and 59% at 2, 4, and 8 days, respectively. This loss of dendritic size is accompanied by the invasion of glial processes and cell bodies into what is normally a glia-free zone containing the NL dendrites. This reaction is noticable by 12 hours post-lesion on the ventral side but is conspicuously absent on the dorsal side at all ages.

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217.9 INJURY AND DENERVATION STIMULATE MUSCLE DEDIFFERENTIATION IN REGENERATING NEWT FORELIMBS. Jo Ann Cameron* and Allen Hilgers* (SPON: C.L. Prosser). University of Illinois College of Medicine and Department of Anatomical Sciences, Urbana, IL 61801.

The dedifferentiation response of transected muscle fibers and of denervated, transected muscle fibers of a stump muscle following limb amputation was characterized. The spatial pattern of neuromuscular junctions was mapped for the anconeus scapularis medialis muscle in the adult Notophthalmus viridescens. The map was prepared from sections of an intact limb cut in the frontal plane. The sections were collected serially, stained for acetylcholinesterase activity (Hardy et al, Neuroscience Letters, 3, 1976), mounted, and stained with hematoxylin and eosin. Projections of each section were traced on transparencies, and the three dimensional configuration of the neuromuscular junction array on several different muscles was analyzed. The anconeus scapularis medialis was chosen for detailed assay because of two clearly definable characteristics: 1) the muscle fibers extend the length of the entire muscle from origin to insertion, and 2) in the frontal plane, the pattern of neuromuscular junctions appears as a diagonal line extending across the muscle in a proximodistal direction. The deep fibers were found to be innervated by the proximal array of neuromuscular junctions, while the superficial fibers were found to be innervated by the distal array of neuromuscular junctions. This pattern was verified in the sagittal plane.

Amputation through the distal one third of this muscle transected and denervated the superficial group of fibers, but left the deeper fibers transected and innervated. Histological examination of the anconeus scapularis medialis muscle was performed at periods from one to fourteen days post-amputation. Sections at days one and three confirmed that the fibers had been transected. By six days post-amputation only the denervated muscle fibers showed signs of dedifferentiation such as disappearance of mature fibers, and an increase in mitotic figures and mononucleated cells. The deeper fibers were intact at six days. At fourteen days post-amputation the area of dedifferentiation had not enlarged and the underlying innervated fibers still appeared intact, indicating that very few additional fibers had undergone dedifferentiation. At this time a blastema had begun to form at the distal tip.

We conclude that injury and denervation of individual muscle fibers is a stimulus for dedifferentiation. We are currently investigating whether our observations hold true for other muscles in the regenerating newt limb.

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217.10 REDUCED INNERVATION DURING AN EARLY SENSITIVE PERIOD PREVENTS DEVELOPMENT OF TASTE BUDS IN THE RAT VALLATE PAPILLA Mark A. Hosley and Bruce Oakley, Neuroscience Lab. Bldg., Div. of Biological Sciences, Univ. of Michigan, Ann Arbor, MI 48109.

It is known from previous work with adult rats that bilateral IXth nerve denervation results in a 100% loss of vallate taste buds, whereas unilateral denervation results in only a 10-12% loss of taste buds, apparently due to bilateral innervation. We have found that large numbers of vallate taste buds never develop when unilateral IXth nerve removal is performed on infant rats.

The right IXth nerve was removed at 3 days post-partum (pp) and vallate taste buds counted in experimental and control rats at ages 5 to 90 days pp. By age 90d normal rats have 625±83 SD vallate taste buds. In contrast, experimental rats developed only 37% of the normal number of taste buds by 90d (230±36). Taste buds were present throughout the papilla, indicating that the effect did not result from an inability of the remaining IXth nerve to reach the taste bud precursor cells. The deficiency in taste bud numbers was further increased if, in addition to removal of the right IXth nerve at 3d, the left (contralateral) IXth nerve was either crushed at the same time (25±27 vallate taste buds) or crushed at 3d and again at 10d (27±39 vallate taste buds). When unilateral denervation was carried out at progressively later ages (0d-20d pp) more taste buds were formed; a mean of 476±94 taste buds formed in animals operated on at 20d and sacrificed at 90d. We propose an early sensitive period in which the nerve fibers and taste bud precursors must interact to ensure the formation of normal numbers of taste buds.

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217.11 SHORT-TERM ACTIVATION OF TYROSINE HYDROXYLASE IN RESIDUAL NORADRENERGIC NERVE TERMINALS AFTER INTRAVENTRICULAR 6-HYDROXYDOPAMINE. A. L. Acheson and M. J. Zigmond. Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260

Intracerebroventricular administration of the neurotoxin 6-hydroxydopamine (6-HDA) produces a rapid and permanent degeneration of norepinephrine (NE)-containing nerve terminals with little destruction of their cell bodies of origin. Thirty-six hrs after 6-HDA (250 µg) was given to adult, male rats, hippocampal NE content was reduced to 23% of the intact control. However, hippocampal tyrosine hydroxylase (TH) activity was only reduced to 86% of control as measured under subsaturating conditions (i.e. at pH 6.6 in the presence of 1.5 mM $6MPH_4$, 75 µM tyrosine). This 3.8-fold increase in TH activity relative to NE content appeared to result from cAMP-dependent phosphorylation of the enzyme. First, while the apparent V_{max} for TH decreased to 20% of intact control, the apparent K_m for pterin cofactor decreased from 3.4 mM to 0.72 mM; this change could be mimicked by incubation of control enzyme under cAMP-dependent protein phosphorylating conditions. Second, while the activity of TH from normal hippocampus declined by 65% between pH 6.2 and 6.6, activity of enzyme from lesioned animals showed no decline over this pH range; nor did normal hippocampal enzyme incubated under phosphorylating conditions. Third, TH activity from lesioned tissue could not be further increased by in vitro exposure to phosphorylating conditions although activity from intact tissue was increased 3.5-fold.

In other experiments the rate of thermal inactivation (50° C) of TH was examined. Normal enzyme pre-incubated under phosphorylating conditions ($T_{½}$ = 2 min) declined faster than enzyme incubated under control conditions ($T_{½}$ = 9 min), whereas enzyme from lesioned animals declined much more slowly ($T_{½}$ = 16 min). However, when an aliquot of normal supernatant was added to supernatant from 6-HDA-treated animals, the rate of the inactivation of the enzyme increased markedly above that of control, an effect which could be inhibited by the phosphatase inhibitor, NaF (100 mM). These results are consistent with the above findings and further suggest the loss of TH phosphatase activity in 6-HDA lesioned tissue.

Since detectable changes in the activation state of hippocampal TH disappeared within 21 days, at which time the apparent V_{max} of the enzyme is increased, we conclude that after 6-HDA induced degeneration of central noradrenergic terminals there is a rapid activation of existing TH molecules within residual terminals which may serve a compensatory role, temporarily increasing the biosynthetic capacity of remaining NE terminals until this can be accomplished by an increase in the amount of available TH enzyme.

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218.1 THE REGENERATION OF SEROTONERGIC FIBERS IN THE ADULT RAT HYPOTHALAMUS:AN IMMUNOCYTOCHEMICAL STUDY. M. Frankfurt and E.C. Azmitia, Dept. of Anatomy, Mt.Sinai Sch.Med., NY, NY, 10029

The regeneration of serotonergic fibers in the adult rat hypothalamus was studied with serotonin (5-HT) immunocytochemistry following unilateral injection of 5,7-dihydroxytryptamine (5,7-DHT) into the dorsolateral hypothalamus. Injections of 5,7-DHT (3ug free base) were made using a glass micropipette with a tip diameter of 50-80 um. Rats were killed by transcardiac perfusion 3,7,12,19,30 and 50 days post lesion. Prior to perfusion the animals were treated with pargyline (200mg/kg) and L-tryptophan (200mg/kg). Vibratome sections (50um) were processed for 5-HT immunocytochemistry using the immunoperoxidase technique.

Three days post lesion there is considerable degeneration of 5-HT fibers ipsilateral to the lesion. Fibers in the medial forebrain bundle (MFB) are swollen and stained darkly for 5-HT. There is little degeneration contralateral to the lesion and this is generally restricted to the area around the fornix. Swollen fibers are evident 3-12 days post lesion and there is a gradual decrease in the density of 5-HT fibers in the MFB. In the medial and periventricular areas of the hypothalamus there are virtually no 5-HT fibers on the injected side 7-30 days post lesion. Sprouts are clearly seen to emerge from the swollen proximal stumps 12-19 days post lesion. Thirty days following the lesion there is a partial reinnervation of the lateral hypothalamus (MFB region) whereas in the medial and periventricular hypothalamic areas no reinnervation is apparent. Fifty days post lesion there is an increase in 5-HT immunoreactivity in the lateral hypothalamus on both the lesion and control sides of rats injected with 5,7-DHT as compared to saline injections. In addition the medial and periventricular areas are reinnervated in these animals.

In order to assess whether this increase in 5-HT immunoreactivity represents a hyperinnervation (50 days post lesion) biochemical studies of 5-HT levels and 3H-5-HT uptake are in progress.

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218.2 A QUANTITATIVE ANALYSIS OF THE EFFECT OF TRIIODOTHYRONINE ON REGENERATION OF CENTRAL CATECHOLAMINERGIC NEURONS. D. W. Hoovler* and B. H. Hwang* (Spon: J. D. Connor). Department of Anatomy, College of Medicine, Pennsylvania State University, Milton S. Hershey Medical Center, Hershey, PA 17033.

Regeneration of catecholaminergic (CA) neurons in the mammalian CNS is known to be especially vigorous following a chemical lesion. The CA neuron terminals innervating the paraventricular nucleus (PVN) of the hypothalamus exhibit substantial, though incomplete, regeneration up to 11 months after lesion with the neurotoxin 6-hydroxydopamine (6-OHDA) (Hwang et al., '80). The thyroid hormone, 3,5,3'-triiodothyronine (T_3) has been shown to promote axonal regeneration in many, but not all studies. To examine the relationship between T_3 and CNS regeneration, the effect of daily administration of T_3 (25 µg/kg, i.p.) on regeneration of CA terminals in the PVN of young adult male Sprague-Dawley rats (n=19) was quantitatively analyzed following lesion by cerebral intraventricular injection of 6-OHDA (200 µg, free base). After T_3 treatment for 21 or 56 days, animals were perfused with a 2% glyoxylic acid solution and 16 µ thick cryostat sections of the PVN were processed for fluorescence microscopy (FM) using the SPG method of de la Torre ('80). Fluorescent CA varicosities in 4 fields of 2500 μ^2 each were counted on 3 micrographs (400X) from each animal. A two-way analysis of variance demonstrated a significant ($p<.01$) increase in the number of CA varicosities/2500 μ^2 in both T_3-treated and control animals after 56 days as compared to 21 days. A significant ($p<.05$) increase in the number of CA varicosities/2500 μ^2 was also present in T_3-treated animals when compared with controls over the 21 and 56 day survival periods.

In an effort to develop a more objective method of quantitation, additional studies were performed to determine the relationship between fluorescence intensity and the number of fluorescent varicosities per unit area. The same tissue sections photographed above for manual quantitation were re-examined directly on the fluorescence microscope with the aid of an automatic exposure camera. The value obtained from the difference in exposure time (secs) between a non-PVN (background) area and a given area of the PVN was used as an index of the number of fluorescent CA varicosities per unit area. A correlation analysis of values obtained by the two quantitative methods produced a significant ($p<.001$) correlation coefficient of $r=.85$.

Quantitative analysis of fluorescent CA varicosities of the PVN has revealed that T_3 significantly enhances central CA neuron regeneration. Further studies are planned to examine this effect at additional survival periods and at the EM level.

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218.3 REGENERATION OF AXONS FROM THE TRANSECTED OPTIC TRACT OF ADULT RATS INTO AND THROUGH NEOCORTICAL TRANSPLANTS D.T. Ross and G.D. Das* (Spon. C.A. Pratt) Dept. of Biol. Sci., Purdue Univ., West Lafayette, Ind.

Although neurons in the CNS of adult mammals may possess the capacity for axonal regeneration following axotomy, connections severed by surgical lesions within the adult CNS are not normally reestablished. However, when embryonic neural tissue is transplanted at the lesion site, this regenerative capacity is expressed as afferent ingrowth to the transplant. The present study was designed to determine how effectively heterotopic transplants could support the regenerative growth of retinofugal axons following surgical transection of the optic tract.

The optic tract of adult (4 months old) female Long Evans rats was transected in either the transverse or the sagittal plane. Immediately following the lesion, 2.5-3.5 mm^3 of 17 day embryonic neocortical tissue was stereotactically transplanted at the lesion coordinates. One to 8 months after transplantation each animal received an intravitreal injection of HRP in the eye contralateral to the transplant.

All transplants which were anatomically integrated with the host brain parenchyma across interface positions with the LGN or optic tract received retinal afferent ingrowth. Transplants exhibited four types of retinal afferent ingrowth: 1) Opposite interface positions with the LGNv and LGNd patches of HRP labelled fibers penetrated $\leq$ 150 µm into the transplant parenchyma; 2) Across some interface positions with the optic tract, fibers or small fascicles of fibers penetrated 250-500 µm and ramified within the transplants; 3) Large fascicles of fibers grew across interface positions with the transected optic tract, coursed through the transplant parenchyma (1-2 mm) and re-entered the retinofugal projection caudally and medially; 4) Large bundles of fibers also grew in from transected portions of the optic tract, traversed the transplant parenchyma along the contour of the transplant (2-4 mm), re-entered the host brain caudally and reinnervated regions of the optic layers in the superior colliculus which had been deafferented by the transection.

The nature and magnitude of regenerative growth from transected rat optic tract depends upon the size and position of the lesion and the positions at which the transplant is anatomically integrated with the host brain parenchyma. The milieu of an embryonic neocortical transplant appears to provide non-specific mechanical and local trophic support for regenerating retinofugal axons.

218.4 APPARENT RECONSTRUCTION OF THE CAUDATE NUCLEUS OF THE RAT WITH CULTURED FETAL NEURONS. D. E. Anderson, H. K. Kulmala, S. A. Lorens. Depts. of Neurosurgery, Neurology, and Pharmacology, Loyola University Medical Center, Maywood, IL 60153.

The development of an animal model for Huntington's Disease through the use of bilateral striatal kainic acid lesions in the rat, has been pursued by several investigators. Morphological and biochemical similarities such as neostriatal neuronal depletion, decreased levels of intrinsic neostriatal neurotransmitters including acetyl choline, GABA and Substance P have been observed. Additionally, axons of passage in, and afferent terminals to the kainate-lesion caudate are left intact. Intracerebral neuronal transplantation in an attempt to reconstruct the damaged neuronal circuitry of the rat caudate has been performed utilizing dissociated fetal CNS tissue suspensions, (Schmidt et al. Br. Res. 218:347, '81). Ultimately, in the application of these techniques to higher mammals, the ability to repair neuronal damage at deep brain sites using readily available dissociated suspensions of cultured neurons would be advantageous. We report the successful transplantation of cultured fetal caudate neurons into adult kainate-lesion striata.

Kainic Acid (KA) was stereotactically injected into the neostriata of adult male Sprague-Dawley rats. The extent of neuronal loss was ascertained in several animals by cresyl violet histology. Caudate nuclei were obtained from rat embryos on the 15th to 17th gestational day. The fetal striata were cultured at 37°C in RPMI 1640 media supplemented with 10% fetal calf serum and antibiotics. After 1-4 days the cultured neurons were aggregated by centrifugation and stereotactically injected in the striata of rats receiving KA lesions 11-14 days prior to surgery. Recipient animals were sacrificed 4-8 weeks after transplantation by perfusion, and the brains were histologically examined to determine graft viability.

Initially, light microscopic evaluation was performed on host brains receiving transplants from fetal caudate neurons cultured up to 96 hours. Large viable neurons with developing processes were noted within the grafts. The success of these preliminary results has prompted further evaluation of such grafts for intrinsic neostriatal neurotransmitters by immunohistochemical techniques.

218.5 GROWTH OF AXONS FROM THALAMIC NEURONS INTO PNS GRAFTS. M. Benfey* and A. Aguayo (SPON: M. Rasminsky). Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Canada, H3G 1A4

In a previous study it was demonstrated that cortical and subcortical neurons in the injured adult rat brain elongate axons into PNS grafts inserted into the cerebral hemisphere (Nature 296: 150, 1982). However, in these experiments, retrograde transport of horseradish peroxidase (HRP) labelled only a few thalamic neurons. Our grafting paradigm was therefore altered slightly to determine whether this failure was due to the location of the graft tip within the brain or whether it reflected an intrinsic inability of thalamic neurons to regenerate.

In nine male Sprague-Dawley rats weighing 260 to 315 g the lateral surface of the skull above the insertion of the zygomatic arch was drilled through to the brain. To reach the thalamus an autologous peroneal nerve segment 17 to 21 mm in length was inserted through this opening to a depth of 7 mm into the cerebrum. Six to thirteen weeks later the distal end of the graft was cut away from the temporalis muscle and a 20% solution of HRP was applied to its stump. Twenty-four or forty-eight hours later these animals were perfused and 40 µm thick sections of their brains were prepared for visualization of HRP labelled neurons (Mesulam, M.M., J. Histochem.Cytochem. 26: 106, 1978). Sections were counterstained with thionin and examined using brightfield light microscopy.

In total 131 labelled neurons were found and these were distributed in layers II to IV of the sensory cortex (22), the basal ganglia (6), and the hypothalamus (9) but the majority (94) were in the thalamus. Of the latter group the most consistent pattern of labelling was found in the reticular nucleus where 74 neurons were labelled in five of the animals. There were also cells in the anterior nucleus (14), the ventral nucleus (4) and in Zona Incerta (2).

These results demonstrate that neurons in several nuclei of the adult rat thalamus are also able to regenerate axons through a peripheral nerve environment.

218.6 REGENERATION AND REORGANIZATION OF THE OLFACTORY SENSORY INPUT FOLLOWING PARTIAL BULBECTOMY. P.P.C. Graziadei and G.A. Monti Graziadei. Florida State University, Tallahassee, FL 32306

Previous work has shown that section of the olfactory sensory axons induces degeneration of their perikarya. From the neurogenetic matrix (basal cells) new neurons are formed and their axons can grow into the cranial cavity where they reinnervate those CNS regions which, following surgery, are more close to the lamina cribrosa (Graziadei, Levine and Monti Graziadei, Neuroscience 4, 713, 1979).

In the present experiments NIH-CD1 mice, 8-10 days old have been unilaterally partially bulbectomized. The surgery has resulted in removal of 1/2 to 2/3 of the rostral portion of the olfactory bulb. The animals have been allowed to survive 60 to 150 days. Twenty animals have been processed for light microscopy observations and the heads, previously fixed, embedded and serial sectioned, have been stained with a rapid silver method (Loots et al., Stain Technol. 58, 97, 1979). Twenty animals, previously perfusion-fixed with aldehydes have been prepared for TEM observations of the main and accessory olfactory bulb.

Silver stain preparations have shown that the sensory axons reinnervate the spared olfactory bulb and form glomerular structures in a number of bulbar regions normally not invaded by the sensory input. Glomerular structures, lacking the periglomerular neuron component, have been identified in the external plexiform layer, in the mitral cell layer and in the granule cell layer. These structures have been identified as being formed by olfactory proper as well as vomeronasal fibers. The ectopic glomerular formations formed by the sensory axon terminals enclose the branching dendritic expansion of the bulbar neurons. With TEM a number of different synaptic contacts have been recognized and a demonstration of them will be provided. The above observations indicate the considerable plasticity of the post-natal bulbar cortex following experimental lesion and the capacity of both the olfactory and vomeronasal input to influence its target neurons in the experimental conditions outlined above.

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218.7 CORTICAL TRANSPLANT CONNECTIONS WITH HOST BRAINS DAMAGED IN UTERO. M.K. Floeter*, L. Yurkewicz and E.G. Jones. James L. O'Leary Division of Experimental Neurology and Neurological Surgery and McDonnell Center for Higher Brain Function, Washington University School of Medicine, St. Louis, MO. 63110.

Rats exposed to the cytotoxic drug methylazoxymethanol (MAM) on fetal day 15 or 16 have a thin cerebral cortex lacking layers II,III, and IV. The loss of layer III neurons results in a greatly reduced corpus callosum, but the loss of layer IV does not prevent ingrowth of thalamic afferents (Jones et al., Dev. Brain Res. 2: 425, 1982).

We wished to investigate whether transplants of fetal cortical tissue (Jaeger and Lund, J. Comp. Neurol. 194: 571, 1980) could "reconstitute" the cortex of MAM treated hosts and salvage some aspects of cortical connectivity. In order to provide a graft marker, fetal donors were labeled with tritiated thymidine on E14 or E15 and used for transplants 2-3 days later. Transplants were of two types: solid grafts, in which a slice of fetal cortex was embedded in the host brain, and suspension grafts, in which freshly dissociated cortical cells were injected into the host brain. Host rats were treated with MAM on E15 or E16, received transplants on the first or second postnatal day, and were allowed to survive for varying times, up to six months.

Solid grafts were most frequently found adjacent to the ventricle or pia mater, often forming a discrete mass. Suspension grafts tended to reaggregate into a few small clumps adhering to the ventricular or pial surface.

Some grafted neurons sent axons to areas of the host brain, as demonstrated by the retrograde transport of horseradish peroxidase (HRP), Fast Blue, or Nuclear Yellow injected into various target sites. These included the opposite cortex as well as subcortical sites such as the thalamus and spinal cord. When the morphology could be identified, retrogradely labeled cells were pyramidal in form. In parts of some graft areas, anterograde labeling of HRP was seen following injections into host thalamus or contralateral cortex, indicating a degree of host projections into grafts. Alternate sections processed for autoradiography confirmed the identity of the grafts.

In Golgi stained material of the grafts, there were a variety of pyramidal and non-pyramidal cells, often greatly distorted and usually disoriented with respect to the host cortex.

Thus, cortical transplants do not "reconstitute" the MAM treated cortex in the sense of reforming missing layers or repopulating the corpus callosum. However, to a very limited extent they can form appropriate connections. The functional significance of this remains to be determined.

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218.8 POSTNATALLY INDUCED FORMATION OF THE CORPUS CALLOSUM IN ACALLOSAL MICE USING CELLULOSE BRIDGES WHICH SPAN THE HEMISPHERES. J. Silver and M.Y. Ogawa*. Dept. of Anatomy, Case Western Reserve Univ., School of Medicine, Cleveland, Ohio 44106.

Developing axons of the corpus callosum of normal mice are guided across the cerebral midline by growing along the upper surface of a bridge-like glial structure or "sling," which forms transiently between the hemispheres. If the "sling" is lesioned at pre-callosal stages, the would-be callosal fibers whirl into massive neuromas (Probst's bundles) adjacent to the longitudinal cerebral fissure. The fibers persist in this location and tortuous configuration throughout subsequent ontogeny (Silver, J., Neurosci. Abs., 7:548,1981). In the present series of experiments on such surgically induced acallosal mice, we asked the following questions. Among the aberrant callosal axons residing in Probst's bundles, do some or perhaps all still maintain a growth potential during prenatal and early postnatal stages? If they do, would the advancing axons move in the proper direction contralaterally provided they are reassociated with a copy of the oriented glial tissue that guides them during normal development?

In one group of C57BL/6J embryos (embryonic day 16) a bridge-like "sling" prosthesis (a D-shaped piece of cellulose membrane filter, Millipore Corp., 0.45µm pore size) was placed immediately into the cerebrum following the initial "sling" lesion (50 embryos). In a second group of acallosal animals the cellulose "sling" was introduced into the brain postnatally (50 embryos on P2 and P4). Our goal in both experiments was to bridge the gap between the Probst's bundles. All animals were allowed to survive until P4 or P9. The brains were embedded in paraffin, sectioned at 10um and stained with silver.

The patterning of "callosal" axons in acallosal animals that also had "sling" replacements was striking in contrast with acallosal animals that were not given implants. Indeed, in acallosal animals with implants, many fibers whose destiny would have been to remain within the confines of Probst's bundles, grew or sprouted out of the bundles, crossed the cerebral midline and in 6 cases continued into the opposite hemispheres. The fibers did not grow on the cellulose itself. Rather, they traveled within a coating of reactive glial cells (probably astrocytes) that encased the bridge implant. We have used a variety of untreated plastics as "control" implants and have failed to redirect axons using these substances. We suggest that the use of such materials is unsuccessful because smooth surfaced plastics do not become coated by reactive cells and, in turn, do not form adequate, growth promoting substrata. Thus, our data suggest that substantial numbers of misplaced callosal axons can be induced to grow contralaterally if they are given the opportunity to interact with a properly aligned, glial coated scaffold. Supported by NIH #NS-15731.

218.9 THE EFFECT OF STRIATAL TARGET NEURONS ON THE MATURATION IN VITRO OF MESENCEPHALIC DOPAMINERGIC NEURONS IS POTENTIATED BY GM_1 GANGLIOSIDE. A.Leon*, R. Dal Toso*, U. Di Porzio*, L. Facci*, S. Mazzari and G. Toffano. Dept. of Biochemistry, Fidia Research Laboratories, 35031 Abano Terme, Italy; Institute of Molecular Biology, University of Naples, 80100 Naples, Italy.

The administration of GM_1 monosialoganglioside to rats with unilateral hemitransection produces an apparent and significant recovery of biochemical, immunohistochemical and behavioral markers for the nigro-striatal dopaminergic pathways (Toffano G., Savoini G., Moroni F., Lombardi M.G. and Agnati L.F., this meeting). In order to have a better insight in the mechanism by which GM_1 elicits the above effect we have used, as a model system, co-cultures of dissociated mesencephalic dopaminergic neurons with striatal target neurons.

Dissociated mesencephalic and striatal neurons are taken from 13 and 15 day old mouse embryos respectively and co-cultured in a serum free hormone supplemented medium (Prochiantz A. et al., Nature, 293:570-572, 1981). In this condition glial proliferation was practically absent. GM_1 was added to culture medium at a final concentration of 10^{-8} to 10^{-6} M and its effects were evaluated both morphologically and biochemically. Such a treatment resulted in increased rate of neurite outgrowth associated with increased acquisition of dopaminergic parameters (^{3}H-dopamine uptake, ^{3}H-dopamine synthesis and TH-related immunofluorescence). Such data strongly support results obtained in vivo and suggest active involvement of GM_1 in regulation of neuronal outgrowth, differentiation and synaptic contact. Similar conclusions have also been suggested by Obata et al. (Nature, 266:369-371, 1977) and by Willinger & Schachner (Dev. Biol., 74:101-117, 1980). Experiments are currently in progress in the attempt to associate GM_1-induced effects with modification of the activity of maturation factor(s), present in culture, on dopaminergic neurons.

218.10 Functional correlates of reactive glial protein synthesis in degenerating rat optic nerve

R.G. Pellegrino, M.J. Politis*and J.M. Ritchie Albert Einstein Col. Med., Bronx, N.Y. and and Yale Univ. School of Med., New Haven Conn.

Glial reactivity following mammlalian CNS injury results in an environment hostile to axonal regeneration. The biochemistry of gliosis is poorly understood. Previous studies in this laboratory showed de novo synthesis of 37K protein(s) distal to site of crush in rat optic nerve 7 and 10 (and to a less extent at 20) days postoperatively, but not in unoperated nerves of in crushed nerves 3 days post-operatively. This protein (tentatively reactive glial protein, RGP) is a putative biochemical marker of reactive (vs. non-reactive) glial tissue.

The present study was undertaken to determine the relationship between initiation of RGP synthesis and axonal degenerative changes in crushed rat optic nerve. Two approaches were taken: (1) De novo protein synthesis was assayed proximal to site of optic nerve crush ten days post-operatively by SDS-PAGE, and (2) the time course of functional axolemma disappearance distal to site of optic nerve crush was assessed by saxitoxin binding.

Results showed no detectable RGP synthesis proximal to site of optic nerve crush. Preliminary data indicate that saxitoxin binding to crushed relative to unoperated nerves is diminished 5 days post-operatively. This is consistent with the widespread axonal degeneration observed by light microscopy between 3 and 7 days post-crush. In summary, these data support the contension that RGP synthesis following CNS injury occurs along with or subsequent to axonal degenerative changes.

218.11 TRANSPORT OF SPECIFIC PROTEINS IN THE GOLDFISH RETINOTECTAL PATHWAY. Wolfgang Quitschke* and Nisson Schechter. Departments of Biochemistry, Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, New York 11794

Molecular components and events associated with nerve regeneration can be studied in the visual pathway of goldfish. In previous studies (Brain Res., 201 (1980) 347-360) involving optic nerve crush, specific proteins of the retinotectal pathway were identified by two-dimensional gel electrophoresis. Two of these proteins whose concentration correlated with the regeneration process were designated as ON_1 and ON_2. They are synthesized in the retina, highly concentrated in the optic nerve and detected in the tectum. They are associated with a particulate fraction, have a molecular weight of 58 K daltons and an isoelectric point of approximately 5.6. They are not associated with myelin, actin or tubulin preparations. In order to further classify these proteins and to gain some insight as to their possible function, a series of experiments were performed to determine their phase of axonal transport.

1 mCi of ^{35}S-Methionine was injected into the right eye of 12 goldfish. One group of goldfish was processed at 12 hrs after injection and another after seven days in order to determine which proteins are associated with fast and slow transport respectively. Right and left optic nerves and tecta were homogenized separately in 0.4% SDS and then precipitated with five volumes of acetone cooled to -20°C. After storage at that temperature for 12 hours the precipitated proteins were centrifuged at 10,000 xg. The supernatant was discarded and the pellet rehomogenized in water and lyophilyzed. The proteins were solubilized in a buffer containing 0.05M Ches (pH 9.0), 2% SDS, 1% DTT and 5% glycerol. Proteins were separated by two-dimensional gel electrophoresis as described by O'Farrell (J. Biol. Chem. 250, (1975) 4007-4021) and visualized by Coomassie blue staining and autoradiography.

The results indicated that proteins ON_1 and ON_2 are only detectable in the slow phase of axonal transport. Proteins in this phase have been implicated as components of the cytoskeletal complex. The cytoskeleton is a possible mediator of cell-cell interactions since it is believed to determine cell shape and in some systems it is continuous with membrane proteins exposed to the cell surface. The relationship of ON_1 and ON_2 to the cytoskeletal complex is currently being investigated.

218.12 CHANGES IN THE CONTROL OF PROTEIN SYNTHESIS UNDERLYING OPTIC NERVE REGENERATION IN GOLDFISH. Michael A. Deaton*, Susan E. Bock*, G. Jack Snipes*, and John A. Freeman. Department of Anatomy, Vanderbilt University, Nashville, Tennessee 37232

We have examined changes in the expression of proteins during regeneration of the goldfish optic nerve utilizing two-dimensional electrophoresis coupled with an ultra-sensitive silver stain (Wray, et al, 1981) and 35S-methionine flourography. Time course studies (0 days through 10 weeks post-crush) reveal distinctive changes in the overall protein composition, and the characteristic appearance of as many as fifteen new growth-associated proteins (GAPs) whose expression is selectively enhanced during different stages of regeneration. Combining flourography with silver staining permits separation of GAPs into sub-categories according to their transport velocities. It also allows a determination of the per cent of total nerve protein devoted to GAPs during the growth state. In addition, the technique permits accurate isolation of proteins of interest. We have further analyzed electrophoretically separated proteins using a computerized quantitative densitometric scanning system. These techniques reveal five distinct polypeptide subsets, ranging from 10,000 to 50,000 daltons and from a slightly acidic pI to a very basic pI, which undergo non-coordinated alterations in synthesis during re-growth of the optic nerve. The most dramatic enhancement of synthesis occurs at two to three weeks in a group of basic proteins with a molecular weight of approximately 50,000 daltons. Furthermore, persistent changes in a set of neutral pI polypeptides with an apparent molecular weight of 30,000 to 40,000 daltons indicate that even at ten weeks post-crush, by which time synaptic contacts have been re-established , protein synthesis has not yet returned to its normal state. The time dependent induction of different GAPs suggests that a characteristic alteration in genetic expression takes place in the retinal ganglion cells during optic nerve regeneration. These characteristic times also indicate a probable role for GAPs in neurite extension and synaptogenesis. Preliminary results from our laboratory and others reveal that goldfish GAPs (Benowitz and Padda, 1981) and toad GAPs (Skene and Willard, 1981) have similar physical properties, suggesting an evolutionarily conserved mode of CNS regeneration in lower vertebrates. Supported by Grant EY01117 from the NEI.

218.13 REGENERATION OF RAT OPTIC AXONS INTO ADJACENT PERIPHERAL NERVE GRAFTS M.J. Politis* and P.S. Spencer*, Inst. of Neurotoxicology, Albert Einstein Coll. of Med., Bronx, N.Y.

Regenerating mammalian CNS axons undergo extensive axonal elongation if provided with a suitable periaxonal environment such as a peripheral nerve. Extensive elongation of regenerating CNS axons is observed in peripheral nerve grafts placed in the vicinity of injured spinal cord or brain (David and Aguayo, Science 214:931, 1981 and Benfey and Aguayo, Nature 296:5853, 1982). The present study was undertaken to determine if axons from the the injured rat optic nerve, a myelinated CNS nerve not capable of regeneration in situ, can regenerate into adjacent grafts of peripheral nerve tissue.

The right optic nerve of Sprague Dawley rats was exposed and a slit made in the dural sheath 1 mm behind the eyeball. Approximately half of the cross-sectional area of the nerve at that level was then crushed with a pair of jeweler's forceps. A segment of peroneal nerve was removed from the leg and inserted into the slit with 9.0 sutures. Grafts were removed 5 weeks later and prepared for microscopic examination to determine the total number of regenerated axons. Innervation resulting from injured peripheral nerves adjacent to the optic nerve lesion was assessed in separate experiments in which retinae (the source of optic axons) were removed two weeks prior to sacrifice.

By five weeks post-operatively the overwhelming majority of fibers in the graft was myelinated. The total number of myelinated axons in grafts from 5 animals in which retinae were not removed ranged between 30 to 400. Between 0 and 10 myelinated fibers were seen in 4 animals in which retinae were removed two weeks earlier.

These results suggest that a small portion of axons from myelinated optic nerves can regenerate if provided with a suitable milieu.

218.14 CHOLESTEROL SYNTHESIS AND NERVE REGENERATION. Anne M. Heacock, Paul D. Klinger*, Edward B. Seguin* and Bernard W. Agranoff. Neuroscience Lab, University of Michigan, Ann Arbor, MI 48109.

In this report, we examine the requirement of cholesterol biosynthesis for goldfish optic nerve regeneration. We have previously observed that the outgrowth of retinal explant neurites in vitro was inhibited by the cholesterol synthesis inhibitor, diazacholesterol (DAC, 10^{-5} M). This inhibition could be reversed by added mevalonolactone (MVA) but not by cholesterol. Here we describe the effect of DAC, administered in vivo, on goldfish retina cholesterol biosynthesis and axonal transport and on recovery of visual function following optic nerve crush.

DAC has been reported to inhibit a final step in cholesterol biosynthesis, the conversion of desmosterol to cholesterol. The effect of intraperitoneally (IP) injected DAC (0.02-0.4 nmoles) on retinal cholesterol biosynthesis was examined 24 h following intraocular (IO) injection of [^{3}H]MVA (6-8 μCi). DAC treatment had no effect on the total radioactivity incorporated into chloroform:methanol soluble material. Separation of the labeled lipids by argentation TLC, however, revealed a greater than 95% inhibition of cholesterol labeling for at least one week following a single IP injection of 0.2 nmoles of DAC. The remainder of the radioactivity was distributed among desmosterol and other more slowly migrating material. While DAC has been reported to inhibit axonal transport, no such effect on transport of [^{3}H]-proline labeled proteins in the goldfish was observed at concentrations that block cholesterol synthesis. To determine if the in vitro growth inhibition serves as a predictor of in vivo effects on optic nerve regeneration, the rate of return of visual function was compared in fish given repeated IP injections of saline or DAC (0.2 nmoles) following optic nerve crush. Recovery was measured by means of a shock-avoidance response. In contrast to the retinal explant results, no striking effects of DAC on optic nerve regeneration were detected. If sterols synthesized in vivo in the presence of DAC can substitute for cholesterol, they should be axonally transported. This possibility was examined in optic tecta removed from control and DAC-treated fish at 10 days following IO injection of [^{3}H]MVA. In control fish, essentially all transported radioactivity co-migrated with cholesterol. DAC treatment had little or no effect on the total amount of labeled lipid transported, however this radioactivity did not co-migrate with either cholesterol or desmosterol but with more slowly migrating substances. (Supported by NIH Grant NS 13743.)

218.15 2D GEL ELECTROPHORESIS OF AXONALLY TRANSPORTED PROTEINS IN REGENERATING XENOPUS OPTIC NERVES. B.G. Szaro*, Y.P. Loh+, and R.K. Hunt*. Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218, +Laboratory of Developmental Neurobiology, NICHD, Bethesda, MD 20205.

The visual system of the South African clawed frog, Xenopus laevis, has long been used as a model system for anatomic and electrophysiologic studies on nerve connections during development and regeneration. In an attempt to correlate molecular changes with those observed anatomically and electrophysiologically, we compared the axonal transport of proteins in regenerating optic nerves to normal nerves using 2 dimensional gel electrophoresis (O'Farrell, 1975). The optic nerves of juvenile (1-6 months post-metamorphosis) frogs were unilaterally severed near the anterior and lateral margins of the optic tectum. Autoradiography by intraocular injection of ^{3}H-proline and electrophysiologic determination of the visual field's projection on to the tectum (1)verified the tecta were denervated, yet the optic nerve from the orbit to the chiasm was left intact for biochemistry, and (2)by 14 days post-lesion, the nerve had begun to regenerate, yet had not reformed its original connections. At this stage of regeneration, animals were intraocularly injected bilaterally with 8-12 μCi's of L-^{35}S-methionine, labeling retinal ganglion cell proteins in both the regenerating (right) and normal (left) eyes of the animal. The optic nerves, eyes, and tecta were then harvested at timepoints appropriate for sampling the fast transport (2 hr & 4 hr), intermediate transport (18 hr), and slow transport (7 days) of retinal ganglion cell proteins through the optic nerve. TCA precipitations done on aliquots of these samples showed a 2-3 fold increase in the amounts of radiolabeled materials in regenerating nerves over normal nerves at every phase of transport. Fluorographic exposures of subsequent 2D gels run in parallel on regenerating and normal samples were compensated for differences in the amounts of loaded radiolabeled material, and were (1)compared visually by analyzing photographic enlargements of homologous gel regions, and (2)measured digitally for select spots using an Optronics image digitizer, a PDP/1160 computer, and 2D gel analysis programs (courtesy of Dr. Merrill, NINCDS). While the labeling of most proteins remained relatively constant, differences were observed in select groups of proteins at each phase of transport. A 57K and a 130K protein in the fast transport phase and a 230K in the slow transport phase were increased. Several proteins decreased during regeneration (16K, 16.5K, & 15K in the intermediate and slow phase, and a 29K protein in the fast transport phase). The identification and roles of these proteins have yet to be established. (Supported by NIH NS-14807 & NSF PCM-8120571 to R.K. Hunt).

219.1 CONNECTIONS OF THE AUDITORY MIDBRAIN IN A TELEOST FISH. Stephen M. Echteler. Neurobiol. Unit, Scripps Instit. of Oceanog. and Dept. of Neurosci., U.C.S.D., La Jolla, CA 92093

In fish the torus semicircularis (TS), a midbrain structure suspected to be homologous to the mammalian inferior colliculus receives not only ascending auditory information but also information from lateral line medullary centers. Recent electrophysiological evidence suggests a parcellation of these sensory modalities within the teleost TS with auditory information relayed primarily to the medial TS and lateral line information to the lateral TS (Knudsen, JCN, 173:417, 1977; Echteler, Soc. Neurosci. Abs. 7:390, 1981).

In the present study Horseradish peroxidase (HRP) was iontophoretically injected into the torus semicircularis of an ostariophysian fish, Cyprinius carpio, following the recording of evoked potentials and multiple unit activity in response to click stimuli. Unilateral injections of HRP into the medial portion of the carp TS coincident with the highest degree of acoustic evoked activity revealed several populations of retrogradely labelled neurons. Within the medulla the anterior octaval nucleus was strongly labelled bilaterally but very few cells were labelled within the nucleus medialis. (Nucleus medialis receives primary input from both posterior and anterior lateral line nerves.) Retrograde cell labelling was also observed in a group of cells surrounding the lateral lemniscus, primarily contralaterally, the ipsilateral superior olive, and scattered cells within the medullary reticular formation bilaterally. Within the ipsilateral optic tectum two populations of cells were retrogradely labelled, fusiform and bipolar, confined to the stratum griseum centrale and the stratum album centrale respectively. Labelled cells were also observed within the nucleus tuberis anterior of the hypothalamus, bilaterally and within the central portion of the medial and lateral lobes of the ipsilateral posterior telencephalon.

Anterograde labelling of fibers and terminals revealed ascending toral efferents. In HRP injections restricted to the medial TS the fibers terminated bilaterally almost exclusively adjacent to the central posterior thalamic nucleus within the caudal diencephalon. With larger HRP injections extending into the lateral TS a second projection into the diencephalon was observed with fiber terminals present over the ipsilateral, large cell region, of the nucleus preglomerulosus. Efferent fibers to the ipsilateral optic tectum were also observed, with some fibers crossing to the contralateral midbrain via the tectal commissure. Commissural fibers were also present: 1) in the ventral tegmentum connecting the two tori in a homotopic fashion, 2) within the posterior commissure, and 3) within the supraoptic decussation.

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219.2 THE PRIMARY SENSORY AND MOTOR CENTERS OF NERVES IX AND X IN A TELEOST. Catherine A. McCormick, Dept. Anatomy, Georgetown Univ., Washington, D.C. 20007

The HRP tracing technique was used to study the sensory and motor centers of the glossopharyngeal and vagus nerves in the pike cichlid, Crenicichla lepidota. Nerves were impaled with an HRP-coated 000 insect pin. Survival times were 4-9 days, and the brains were processed according to the method of Mesulam (1978).

In Crenicichla, the medullary visceral sensory column is modest in size compared to that of cyprinids and silurids. The rostralmost portion of this cell column contains the facial lobe. The vagal lobe has its rostral boundary further caudally, and lies medial to the facial lobe. The caudal boundary of the facial lobe lies at a level near the rostral border of the branchiomeric motor components of nerve IX, whereas that of the vagal lobe is immediately rostral to the obex. Three cellular laminae are present dorsolaterally in the caudal portion of the vagal lobe, although they are not present at its most caudal levels.

Visceral sensory fibers of the vagus nerve terminate throughout the vagal lobe with the exception of its most rostral portion, and have an especially heavy projection to the three cellular laminae. In addition, a small branch of nerve X contributes fine terminal to the facial lobe. This branch joins nerve X proximal to its ganglion. From this point it courses rostral and dorsal and soon bifurcates into rostral and caudal branchlets. The caudal branchlet emerges from the skull superior to the inner ear and its central connections appear confined to the facial lobe.

The glossopharyngeal nerve terminates in the middle two-thirds of the vagal lobe. It supplies a small number of fibers to the laminated portion of this lobe. No terminals were seen in the facial lobe.

Somatic sensory fibers of nerves IX and X ascend and descend in the spinal trigeminal tract and terminate in the spinal trigeminal nucleus and, in the case of nerve X, in the substantia gelatinosa-funicular complex (Herrick, 1907). The somatic sensory component of nerve IX is extremely small.

The motor cells which were retrogradely labeled after injections of nerves IX and X form a continuous column situated dorsolateral to the MLF. Cells in the rostral one third of this column contribute axons to nerve IX; these axons form a genu before coursing through the medulla to exit the brain. Axons of the caudal motor cells are components of nerve X. The branchiomeric motor cells of nerves IX and X are distinct from the efferent cells of the lateral line and eighth nerves, which lie more rostrally and differ in morphology.

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219.3 RELATIONSHIPS BETWEEN TECTUM OPTICUM AND NUCLEUS ISTHMI IN TELEOSTS: MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL ASPECTS. H. Vanegas, B. Williams* and N. Hernández*. Inst, Venezolano de Investig. Científicas (IVIC), Caracas 1010A, Venezuela.

The nucleus isthmi (NI) of non-mammals is homologous to the parabigeminal nucleus of mammals. In teleosts, NI is located in the brainstem tegmentum, receives a projection from the large pyriform neurons of the tectum opticum (TeO) and from the nucleus pretectalis, and in turn sends a projection -apparently only- to the stratum griseum centrale of TeO. NI neurons have extremely broad visual receptive fields and their response to moving visual stimuli is a function of stimulus velocity. NI neurons discharge before and during ipsiversive eye movements elicited by vestibular stimulation in darkness. Electrical microstimulation of NI results in saccadic and nystagmic eye movements.

The present study was carried out in the teleosts Perca, Eugerres and Holocentrus. Neuronal geometry of NI was investigated in Bodian, Palmgren and Golgi preparations and in 1µm plastic sections. In addition, field and intracellular potentials elicited in NI by electrical stimulation of TeO were studied. The somas of NI neurons, ca. 17x13µm in size, are clustered in the NI periphery. Thick (ca. 10µm) dendritic stems emerge from these somas and branch profusely in the NI center or neuropil, where the tecto-isthmic fibers terminate. TeO stimulation elicits a field potential burst of 4-5 spikes at the neuropil (i.e., dendrites). The first of these spikes seems to be an admixture of antidromically elicited potentials -in some neurons- and potentials elicited through electrotonic tecto-isthmic synapses -in other neurons. The relative contribution of these two factors can be varied by electrophysiological manipulations. The other spikes must be elicited through chemical synapses: they show facilitation at low, and blockade at high, stimulus frequencies. Intracellular potentials consist of a burst of spikes similar in pattern to the field potentials. The first spike can follow stimulus frequencies up to 400/sec; the other spikes cannot follow frequencies of 50/sec or more. Given the similarity of field (population) and intracellular (unitary) potentials, the possibility of low resistance coupling between NI neurons was investigated: injection of Lucifer Yellow into one neuron results in labelling of about 20 neurons. In conclusion, activation of tecto-isthmic afferents excites dendritic processes at the NI neuropil through both electrotonic and chemical synapses. The ensuing action potentials occur synchronouly in a large number of neurons, probably due to electrotonic coupling.

219.4 CEREBELLAR AFFERENTS IN THE LONGNOSE GAR (HOLOSTEI). R. Glenn Northcutt. Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109

Afferent pathways to the corpus of the cerebellum of the longnose gar (Lepisosteus osseus) were determined by unilateral innoculation of the corpus (10 cases) with HRP (Sigma VI) paste on the tip of a "000" insect pin. Following survival times of 4-10 days at 24-27°C, the animals were reanesthetized and perfused transcardially with cold phosphate buffer (pH 7.4) followed by 4% glutaraldehyde in phosphate buffer. Brains were removed, embedded in 25% gelatin, and cut at 40µ. Sections were reacted with o-dianisidine or tetramethyl benzidine following modified protocols of Coleman et al., 1976 or Mesulam, 1978.

Unilateral cerebellar injections retrogradely labeled cells in the following forebrain nuclei: ipsilaterally in the caudal entopeduncular nucleus, ventrolateral thalamic nucleus, accessory optic nucleus, nucleus of the ventral optic tract, pars magnocellularis of the superficial pretectal nucleus, central pretectal nucleus, and pars ventralis of the periventricular pretectal nucleus. At midbrain levels, retrogradely labeled cells were seen ipsilaterally in nucleus isthmi, lateral tegmental nucleus, and lateral nucleus of the valvula, and bilaterally in locus coeruleus. At more caudal levels, retrogradely labeled cells were seen contralaterally in the inferior olivary nucleus and the ventral horn of the spinal cord. Larger neurons located immediately beneath the pial surface of the medulla at the level of the inferior olivary nuclei were also labeled bilaterally, as were cells of the descending octaval nuclei and the nuclei of the descending trigeminal tracts. Finally, retrogradely labeled cells were also observed in the inferior raphe nucleus.

Analysis of these results indicates that the cerebellar corpus of gars probably receives extensive visual inputs via the pretectum as well as the optic tectum. Tectal projections to the corpus of the cerebellum are not direct but occur via the lateral nucleus of the valvula and nucleus isthmi. The single largest source of afferents to the cerebellum occurs via the lateral tegmental nucleus whose afferents are presently unknown. In addition, the corpus receives spinal, trigeminal, and vestibular inputs as in other vertebrates.

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219.5 FUNCTIONAL CONNECTIONS OF THE MEDULLARY ELECTRORECEPTOR NUCLEUS OF THE SKATE. D. Bodznick and A.W. Schmidt*. Biology Department, Wesleyan University, Middletown, CT. 06457.

In an attempt to understand CNS processing of electrosensory information in elasmobranchs we have used HRP, evoked potential and single unit techniques to examine details of the afferent and efferent connections of the medullary dorsal octavolateral nucleus (DON) in the skate Raja erinacea. DON is the primary target of electroreceptor afferents which are carried in the dorsal root of the anterior lateral line nerve (ALLN) (Koester, Ph.D. dissertation, U. Delaware, 1981; Bodznick and Northcutt, 1980).

Transganglionic HRP transport in individual rami of ALLN revealed a somatotopic arrangement of electroreceptor terminals in DON. The external mandibular ramus which is the largest in skates and innervates electroreceptors on the pectoral fins and caudalmost portion of the head projects to a large dorsal region of DON. Its rostro-caudal extent includes the caudal 3/4 of the nucleus. The superficial ophthalmic ramus innervating electroreceptors on the snout projects to the most ventral portion of the rostral 2/3 of DON and the buccal ramus innervating electroreceptors intermediate in position on the head terminates in an intermediate position (d-v, r-c) in the nucleus. This separation has been confirmed by recording responses of single DON units to weak local electric fields. Most DON cells receive convergent excitatory inputs from several (ca. 3-6) ipsilateral electroreceptor organs. Weaker excitatory or inhibitory contralateral inputs are apparent after transection of ipsilateral ALLN and are attributable to commissural connections known to exist between the two nuclei (Boord and Northcutt, JCN, in press).

Additional afferent and efferent connections of DON were examined following unilateral injections of HRP into DON. In confirmation of Fink Heimer degeneration and amino acid transport studies (Boord and Northcutt, JCN, in press), efferent connections of DON were to contralateral DON and bilaterally (heaviest contralaterally) to optic tectum and the lateral mesencephalic nucleus (LMN). Many DON units can be driven antidromically by electrical stimulation of the contralateral LMN and evoked potential responses are recorded from LMN and optic tectum to weak electric field stimuli.

Afferent connections of DON revealed by anterograde transport of HRP were from cells of both the central zone and the Purkinje-like cell layer of the contralateral DON. In addition, HRP-filled cells were found scattered along the course of the lateral line lemniscus bilaterally.

219.6 CONVERGENCE OF VISUAL AND ELECTROSENSORY INPUT IN THE TECTUM OF A GYMNOTID FISH. E. Sas and L. Maler. Dept. of Anatomy, Fac. of Health Sci., Univ. of Ottawa, Ont. K1N 9A9, Canada. SPON: Walter Hendelman.

A Golgi analysis has been undertaken to study the vertical and horizontal organization of the optic tectum, to comprehend the mode in which multisensory information may be processed and its functional implications. Although Eigenmannia has small eyes and depends largely on the electrosensory system for orientation, its optic tectum is a well defined six layered structure presenting a segregation of cell types and afferents in a laminar fashion. HRP injections into the eye, torus semicircularis (T.S.d.) and nucleus praeeminentialis (N.P.d.) offered information of the arrangement of these tectal afferents. Retinal fibres were seen mainly within the stratum opticum (S.O.) and stratum fibrosum and griseum superficial (SFGS). Electrosensory information from T.S.d. and N.P.d. project to the deep layers of the optic tectum in topographic register with the retinal projections (Carr et al., '81). Particularly interesting was the observation in Golgi material of: a) Cells with two symmetrical dendritic fields receiving topographically corresponding visual and electrosensory information. b) Cells with one dendritic tree spreading symmetrically in an electrorecipient layer; and a second visual recipient dendrite oriented exclusively towards one side. In such case the topography of the visual and electrosensory maps will not be in complete register, but slightly shifted in relationship to one another. Cells with these two types of electrophysiological responses have been recorded by Bastian in Eigenmannia (Neurosc. Abstr. 1981). Cells with wide dendritic arbors have been noticed in the (SGC) Stratum Griseum Centrale; this correlates well with physiological reports of increased sizes of movement fields within deeper layers. We have found several possible morphological substrates for visuo-electrosensory integration: 1) Dendrites of deep electrosensory recipient cells entering superficial layers where visual afferents predominate. 2) Axons of superficial cells ramifying in deep layers. 3) Intrinsic axons of deep cells reaching visual target neurons. Examples of each of these cases will be presented and discussed.

219.7 CONNECTIONS OF THE LATERAL LINE LOBE (N. MEDIALIS) IN GOLDFISH, CARASSIUS AURATUS. Thomas E. Finger. Dept. Anatomy, Univ. Colorado Health Sciences Center, Denver, CO 80262.

Although much is known about connections of primary lateral line nuclei in electroreceptive teleosts, very little has been reported regarding central connections of lateral line nuclei in non-electroreceptive teleosts. In the present study, HRP was used as an anterograde and retrograde tracer for connections of the primary lateral line nucleus (n. medialis) in goldfish. Injections were made in the n. medialis, torus semicircularis and optic tectum. Following postinjection survival times of 4-10 days, the fish were perfused with 4% glutaraldehyde, and following sectioning, the brains were reacted by modified Hanker-Yates and/or TMB methods.

Three major structures provide input to n. medialis: lateral line ganglia, the caudal lobe of the cerebellum, and n. praeeminentialis. The n. praeeminentialis in goldfish is a collection of small neurons lying lateral to the trigeminal motor nucleus. Strands of cerebellar granule cells run from the n. praeeminentialis, medially to the eminentia granularis, and dorsally to the lobus caudalis. Numerous granule cells of the ipsilateral caudal cerebellar lobe are retrogradely labeled following HRP injections in n. medialis. These cells probably give rise to the bulk of the cerebellar crest. A few larger cerebellar neurons are also retrogradely labeled following these injections.

The n. medialis projects to four principal targets: the caudal part of the cerebellum, n. praeeminentialis, torus semicircularis and deep layers of the optic tectum. All projections to the brainstem are bilateral but are heaviest to the contralateral side. As confirmed by retrograde labeling following tectal injections of HRP, the direct projection to the optic tectum arises from crest cells in n. medialis. In the limited series of injections obtained to date, the medialis-tectal projection is heaviest to the rostral and lateral aspects of the optic tectum.

Following injections into the n. medialis, numerous labeled fibers can be traced into the cellular layers of the contralateral n. medialis. The source of this apparent commissural connection does not appear to be the n. medialis itself since no retrogradely labeled neurons occur in the n. medialis contralateal to the injection.

In summary, with the exception of the tectal projection, the connections of the primary lateral line nuclei are similar in electroreceptive and nonelectroreceptive teleosts. In electroreceptive teleosts, the n. praeeminentialis is a massive structure providing feedback onto the lateral line lobes; in goldfish, the n. praeeminentialis is a relatively minor collection of cells which probably serve the same function. Accordingly, evolution of electroreceptive pathways in the CNS did not involve invention of new structures, but modification of existing neural networks.

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219.8 THREE TYPES OF POSTERIOR LATERAL LINE EFFERENT NEURONS IN LARVAL ZEBRAFISH. W. K. Metcalfe* and C. B. Kimmel (SPON: S. D. Hauschka). Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

Three types of lateral line efferent neurons were revealed in larvae of the zebrafish (Brachydanio rerio) five days after fertilization by applying HRP to lesions of the posterior lateral line nerve, usually at the level of the fourth myotome. All three types are found ipsilateral to the lesioned side, and can be distinguished from one another on the basis of their positions, axon pathways, and dendritic morphologies.

The caudalmost type of cells, rhombencephalic efferent neurons to the lateral line or RELL cells, are located in the hindbrain medial reticular formation. The somata are adjacent to the descending axon of the Mauthner neuron. RELL cells have long and branching dendrites which extend bilaterally into ventrolateral neuropil. The axon courses laterally to exit the brain with the root of the posterior lateral line nerve. There are only one or two RELL cells labeled on each side.

The second type of efferent neuron, rhombencephalic octavolateral efferent neuron, or ROLE cell, is located immediately rostrad of the RELL cells and is also adjacent to the axon of the Mauthner neuron. The ROLE cell dendrites are short and restricted to the local neuropil. The axon courses rostrally to the level of the Mauthner cell body, then laterally to exit with the eighth nerve. The axon then branches, one branch extending into the posterior lateral line nerve and the other to the saccular macula. We observe only one labeled ROLE cell on each side of the hindbrain.

Finally, diencephalic efferent neurons to the lateral line, or DELL cells, are present in the region of the posterior tubercle. Their dendrites branch extensively in the local neuropil. The axons course caudally in the CNS at least to the level of the third somite. The axons send out fine branches at several levels during their caudal course and one of these enters the posterior lateral line root with the RELL axons. About four DELL cells are present on each side.

Published reports suggest a large number of efferent neurons comprising a single class (cf. Bell, C. C., J. Comp. Neurol., 195, 1981). Instead we find three classes of efferent neurons represented by only a few cells each. Although the functional significance of these findings is not clear, especially with respect to the previously unreported diencephalic group, they do indicate that lateral line activity may be modulated by a more diverse array of efferent neurons than was previously known.

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219.9 RETICULAR INTERNEURONS WITH T-SHAPED AXONS IN EMBRYOS OF THE ZEBRAFISH. C. B. Kimmel, W. K. Metcalfe* and E. Schabtach*. Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

A class of interneuron was identified in embryos and young larvae of Brachydanio rerio which may function during fast-start behaviors initiated by the Mauthner neuron. The cells were labeled with HRP applied to lesions of their axons within the brain. The somata are located laterally in the reticular formation of the caudal hindbrain. The proximal segments of their axons project medially to cross the midline, and bifurcate to form rostral and caudal segments which course within the medial longitudinal fasciculus. The T-shaped axons of these neurons distinguish them from previously described interneurons in the zebrafish (Kimmel, C. B. et al., J. Comp. Neur., 205: 112, 1982).

We have observed 8-10 of these neurons, present in a rostrocaudal series, on each side of the brain. However, in most experiments fewer cells are labeled. Cells located caudally in the set are fusiform in shape and possess thin axons. Two very prominent cells are located rostrally in the set on each side. Their somata are round, their dendrites short, and their axons are of large diameter, second in size only to the Mauthner axon. They are identifiable by the second day after fertilization. The rostralmost cells receive axo-axonal synapses from each Mauthner axon, as well as from other unidentified axons in the medial longitudinal fasciculus. Under EM these contacts are seen to be Type I chemical synapses. Both rostral and caudal segments give rise to laterally directed collaterals, which thus project exclusively contralateral to the somata. A large collateral of the rostral segment projects to the trigeminal motor nucleus, and the rostral segment itself forms a distinctive hook-shaped ending within the oculomotor nucleus. The caudal segment forms terminal arbors near the bulbo-spinal junction. Motoneurons at this location innervate musculature of the pectoral fins.

A number of these morphological features are similar to those of the previously described "giant fiber" cells of the adult hatchetfish (Model, P. G. et al., Brain Res., 45: 288, 1972), which receive input from the Mauthner neurons and mediate bilateral responses of the pectoral fins. We propose that the rostral interneurons described here are homologous. It is known also that cranial musculature is bilaterally excited during Mauthner cell-initiated behavior (Diamond, J., in Fish Physiology, 5: 265, 1971). Such responses may be mediated by the rostral axonal segments of these interneurons. (Supported by NIH grant NS 176963.)

219.10 SACCULAR NERVE INPUT TO THE LATERAL DENDRITE OF THE GOLDFISH MAUTHNER CELL: A COMBINED ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL STUDY. Jen-Wei Lin*, Malcolm R. Wood*, and Donald S. Faber. Div. Neurobiology; Dept. Physiology; SUNYAB, Buffalo, NY 14214.

The startle response mediated by the teleost Mauthner cell (M-cell) can be evoked by auditory stimuli; the distal regions of the lateral dendrite of this medullary neuron are innervated by the primary auditory afferents classified as large myelinated club endings. The responses of the goldfish M-cell to the sinusoidal auditory stimuli (400 to 1000 Hz) have been recorded intracellularly. Evoked excitatory postsynaptic potentials (EPSP's) exhibit multiple fast rising components superimposed on an underlying slower depolarization. The amplitude of the fast components was maximal at distal dendritic recording sites, suggesting they are evoked by activity in the large club endings. In contrast, the spatial profile of the slower depolarization suggested it was due to a more distributed input extending to the proximal dendrite as well. Lesion studies indicated that both inputs are mainly mediated by the saccular branch of the eighth nerve. We therefore studied the distribution of its inputs to the M-cell by application of horseradish peroxidase (HRP-type VI) at a distance 1.5 to 2 mm from the medulla. Stained club endings of various sizes terminate on the distal two-thirds of the lateral dendrite, with the largest ones being localized at the center of this region. Some large club endings issue secondary processes from their terminal regions. These processes are less than 10 µm long and end abruptly as spherical swellings, approximately 2 µm in diameter. Similar terminal branching patterns have been observed in electron microscopic studies of material not treated with HRP. A second group of filled terminals, corresponding to the endbulbs of Bodian (J. Comp. Neurol., 68:117-159, 1937), are distributed over the same area, thus being intermingled with the club endings. These endbulbs, presumably excitatory, are in highest density in the proximal and distal most regions of the lateral dendrite. There are no apparent saccular inputs to the cell's soma and axon cap region. Localized HRP injections have been used to determine whether inputs to different regions arise from branches of individual afferents. Fibers giving rise to the endbulbs branch within 50 to 150 µm from the lateral dendrite, but we have not observed more than one secondary process projecting to the M-cell. The localized HRP injections frequently revealed a pattern of afferents terminating on a restricted area of the lateral dendrite, suggesting neighboring fibers in the saccular nerve project to the same region of the target cell.

The use of HRP has revealed a second class of afferent terminals which might mediate the slower EPSP's evoked by auditory stimulation.

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219.11 ELECTRORECEPTIVE AND MECHANORECEPTIVE AFFERENTS OF THE TORUS SEMICIRCULARIS IN THE NOTOPTERID FISH, XENOMYSTUS NIGRI. M. R. Braford, Jr. Dept. of Anatomy, Georgetown University, Washington D.C. 20007

In teleostean fish the principal medullary target of the mechanoreceptive afferents of the lateral line system is nucleus medialis (N. Med.). Electroreceptive teleosts have an additional medullary primary lateral line center that receives input from the electroreceptors--the electrosensory lateral line lobe (ELLL), also known as the posterior lateral line lobe and the lateral line lobe. The African knifefish, Xenomystus nigri (family Notopteridae), possesses both a N. Med. and an ELLL (Braford, Neurosci. Lett., in press) and is electroreceptive (Bullock and Northcutt, J. Comp. Physiol., in press). In Xenomystus, N. Med. and ELLL both receive input from the posterior and anterior lateral line nerves. The projections of these two lateral line centers to the torus semicircularis (TS) were studied with horseradish peroxidase (HRP) and degeneration tracing techniques.

Following HRP injections into ELLL, the majority of the labeled fibers decussate in the medulla and ascend in the lateral part of the contralateral lateral lemniscus. In the midbrain these fibers sweep dorsolaterally to enter the TS where they terminate densely in two adjacent centrally-located regions, termed here nucleus lateralis and nucleus medialis ventralis (after similar regions in the mormyrid TS, Bell et al., Exp. Brain Res., 42, 1981). A substantial number of ELLL efferent fibers course ipsilaterally through the medulla (in a more dorsal position than the crossed ones) and terminate in a similar, but less dense, fashion in the ipsilateral TS.

Following HRP injections into the rostral portions of N. Med., the majority of the labeled axons decussate and enter the more ventral part of the contralateral lateral lemniscus. These fibers ascend to the TS and terminate in two nonadjacent regions, nucleus dorsomedialis and nucleus ventralis posterior, which lie dorsal and ventral to the electroreceptive afferents, respectively. Uncrossed fibers terminate less densely in the same regions of the ipsilateral TS. The electroreceptive and mechanoreceptive zones appear to be entirely nonoverlapping.

This pattern of organization of inputs and the nuclear, rather than laminar, appearance seen in the cytoarchitecture of the TS are comparable to, but less elaborate than, those seen in mormyrids--to which the notopterids are closely related--and differ from those seen in the electroreceptive ostariophysines.

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219.12 PRIMARY PROJECTIONS OF THE LATERAL LINE NERVES IN THE NORTHERN SILVER LAMPREY. Mark C. Ronan* and R. Glenn Northcutt (SPON: M. S. Northcutt). Neurosciences Program and Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

The central projections of the lateral line nerves in adult northern silver lampreys, Icthyomyzon unicuspis, were examined by silver staining of degenerating axons. Under MS222 anesthesia, the ganglion of the anterior (ALLN) or posterior (PLLN) lateral line nerve was unilaterally exposed and lesioned with a heated insect pin. Animals survived three, five or ten days at 14-20°C. Brains were processed according to the Wiitanen modification of the Fink-Heimer technique. The ALLN possesses dorsal and ventral roots. Dorsal root primary afferents project to the electroreceptive dorsal octavolateralis nucleus (DON) and terminate ipsilaterally throughout the longitudinal extent of the neuropil lateral to the periventricular cell plate. Ventral root primary afferents terminate ipsilaterally in the neuropil of the mechanoreceptive medial octavolateralis nucleus (MON) along its full rostral-caudal extent. Rostrally, degenerating afferents extend beyond the anterior border of the MON into the cerebellum and run bilaterally in the cerebellar molecular layer. PLLN afferents enter the brain as a single root and project to the entire longitudinal extent of the ipsilateral MON neuropil. Additionally, some PLLN afferents proceed rostrally into the cerebellum, cross the midline in the cerebellar molecular layer, and then turn caudally to terminate in the dorsolateral neuropil of the contralateral MON.

In lampreys, a major ALLN component, the recurrent collateral (RC), passes caudally around the otic capsule and joins PLLN branches to form a lateral line nerve trunk descending along the spinal column. Central projections of primary ALLN afferents in the RC were determined with HRP histochemistry. Following unilateral RC transection, a gelfoam plug saturated with 40% HRP (Sigma VI) was placed in the lesion. Animals survived ten days at 14-20°C. Transverse sections of the head, 40µ thick, were processed using a modified Mesulam ('78) TMB protocol. Transganglionic transport of HRP resulted in heavy labeling of the ipsilateral ALLN dorsal root and DON; HRP-labeled fibers were also present in the lateral line trunk. The majority of RC thus appear to be electroreceptive afferents supplying regions caudal to the head. As expected, placement of HRP into the transected lateral line trunk at rostral cord levels resulted in labeling of the ipsilateral RC, ALLN dorsal root, and DON. The ipsilateral PLLN was also labeled, confirming the bilateral primary PLLN projections to MON.

Primary lateralis projections in silver lampreys resemble those in sturgeons and elasmobranchs. However, PLLN projections to the contralateral MON and a RC carrying ALLN afferents likely innervating trunk electroreceptors have thus far only been reported in lampreys. Supported by NIH Grant EY02485 to RGN.

219.13 ELECTRON MICROSCOPY OF BRAIN STEM PROJECTIONS TO THE CAUDAL NEUROSECRETORY SYSTEM: HRP TRACER STUDIES. K.E. Miller*, J.P. O'Brien* & R.M. Kriebel*(SPON: J. Fiekers). Department of Anatomy & Neurobiology, The University of Vermont, College of Medicine, Burlington, Vermont 05405.

Recently, we have shown that two nuclei in the brain stem project to the neurons in the caudal neurosecretory system of the molly (P. sphenops). Using light microscopic procedures, HRP-labelled neurons were located in the reticular nucleus of the medulla (RNM) and in the nucleus of the medial longitudinal fascicle (NMLF). To further develop the unique features of the caudal system as a preparation for studies on synaptic control of vertebrate neurosecretory cells, it is necessary to determine the neurochemical nature of the cells which form the descending projection. In other species catecholaminergic cells have been identified in the region of the RNM. Ultrastructural examination of the midbrain NMLF showed that neurosecretory cells were in the immediate proximity of NMLF neurons. The present study was undertaken to examine the ultrastructure of the cells in the RNM and NMLF that project to the caudal neurosecretory system to determine the neurochemical nature of these cells. Fourteen animals were anesthetized with MS222 and the appropriate spinal cord levels exposed through small lateral incisions. Implants of HRP/acrylamide (15% HRP) were placed in the spinal cord between the ninth and third terminal vertebral segments. Animals survived 60 h; brains were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer; transversely sectioned with a tissue chopper at 100μ. The tissue was processed for electron microscopy by either the DAB procedure (J. Adams, per. comm.) or the glucose oxidase-DAB procedure (Itoh et al., Br.Res. 175:341, 1979). Electron dense reaction product was located in small and large membrane-bound profiles. Cells in the midbrain NMLF containing HRP-filled profiles were located bilaterally close to the midline and just beneath the subependyma along the tectal ventricle. These cells were located ventral to the neurosecretory cells and did not contain elementary neurosecretory granules. No HRP reaction product was found in the neurosecretory cells of the midbrain. Cells containing HRP-labelled profiles were also seen in the RNM. The labelled cells in the RNM and NMLF received axosomatic synapses. Numerous cells which were not labelled surrounded the HRP positive neurons in both brain stem nuclei. The innervation of caudal system neurons appears to originate from nuclei which may have heterogeneous projections. The ultrastructural features of labelled neurons have not made it possible to determine their neurochemical nature. Combined fluorescence for neurotransmitters and retrograde tracer studies are in progress.
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219.14 CENTRAL CONNECTIONS OF THE INNER EAR IN A CICHLID FISH. Gloria E. Meredith and Ann B. Butler. Dept. of Anatomy, Georgetown Univ., Washington, D.C. 20007.

Central connections of the sacculus, utriculus, lagena and semicircular canals were studied using HRP and degeneration in the oscar, Astronotus ocellatus. Eighth nerve branches had HRP applied to their cut ends or were severed proximal to their ganglia in animals anesthetized with MS-222 and cold. After survival of 6-15 days, animals were sacrificed, perfused, and the brain processed according to the method of deOlmos and Heimer (Neurosci. Lett., 1977) for HRP or the Wiitanen (Br. Res. 14:546-548, 1969) method for silver impregnation.

Eighth nerve fibers from the inner ear enter the brain laterally, divide, and course rostrally and caudally. Comparison of individual endorgan inputs within each of the octavus nuclei was made across cases with reference to the position of either the entrance of the fifth nerve or of the dorsal border of the descending tract of V. Projections from the utriculus and semicircular canals were found in all five of the octavus column nuclei--anterior, magnocellular, tangential, descending, and posterior. Sacculus and lagena each project to all these nuclei except tangential. Rostralmost projections from the inner ear were traced into the cerebellum--to the eminentia granularis and to the more medially situated granular layer of the corpus cerebellum. Sparse terminal fields were also found in the medial reticular formation.

Utricular and semicircular canal projections consistently overlap in all nuclei. Canals appear to have their heaviest input to the tangential and descending nuclei, whereas the utricular input appears uniform in density among all five nuclei. Utricular terminals, while overlapping canal terminals, were also found in areas free from canal input.

Semicircular canals and utricular projections overlap with the sparser saccular and lagenar terminals in the anterior nucleus. In the magnocellular and descending nuclei, however, a distinct laminar pattern of input is evident: densest saccular and lagenar projections lie dorsal to utricular and canal terminal fields, with some overlap of the borders of each terminal field. At the level of the magnocellular nucleus, a small group of round to fusiform cells lies at the medial edge of the descending tract of V; it receives projections solely from the canals and utriculus and appears to be the rostral pole of the descending nucleus. Otolith endorgan projections overlap in the posterior nucleus.

The efferent cell soma lie medially in a rostrocaudal plane at the level of entrance of eighth nerve fibers.

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219.15 FUNCTIONAL COMPONENTS OF THE FROG LINGUAL NERVE. F. A. Kutyna, J. W. Patrickson* and H.J. Bryant. Dept. of Physiology, Uniformed Services University, Bethesda, MD 20184.

The 2-deoxy-D-[^{14}C] glucose (2DG) method for functional activity was used to study the contribution of components of the Autonomic Nervous System and Central Nervous System to the lingual branch of the frog glossopharyngeal nerve (IX). Combined with horseradish peroxidase (HRP) and Golgi (G) tracer methods this provided a comprehensive view of the neural elements functionally linked to the tongue via the IX nerve.

2-Deoxyglucose The left lingual branch of the cut IX nerve was electrically stimulated at its central stump for a period of 45 min following intracardiac injection of 2DG glucose. Autoradiographs were made from cryosections of the brain, sensory pneumogastric and first sympathetic ganglia. These sections were then stained for histological confirmation. Analysis of the autoradiographs using a microdensitometer constructed from a compound microscope showed high levels of 2DG uptake in the glossopharyngeal (IX) division of the pneumogastric ganglion. There was no observable increase in uptake of 2DG in the vagal (X) portion of this sensory ganglion.

Increased 2DG uptake was seen in the ipsilateral nucleus of the solitary tract. The ipsilateral motor nucleus of IX was labeled by 2DG as was the cephalad pole of the ipsilateral first sympathetic ganglion.

Horseradish Peroxidase HRP powder was applied to the cut central stump of the lingual IX nerve. The brain and ganglia were removed and developed for HRP. The greatest number of labeled cells were found in the ipsilateral IX division of the pneumogastric ganglion. A few cells were labeled in the ipsilateral motor nucleus of IX and the cephalad pole of the ipsilateral first sympathetic ganglion. No cells bodies were found labeled in more rostral brain nuclei.

Golgi Method Frog brains subjected to G staining showed neurons of the nucleus of the solitary tract ascending to higher levels of the brainstem. Neurons of the motor nucleus of IX send processes toward the root of the IX nerve. Small reticular fibers from other levels of the brainstem were found in proximity to these IX nerve cells.

The three methods used in this study (2DG, HRP, G) present evidence for the organization of the neural components of the frog taste system into 3 functional divisions: (1) a primary sensory afferent with cell bodies in the IX division of the penumogastric ganglion; (2) a sympathetic efferent with post-ganglionic cell bodies in the first chain ganglion; (3) a visceral motor component with cell bodies located in the area of the IX motor nucleus. (Supported in part by USUHS Grant C07606)

219.16 FOREBRAIN PROJECTIONS TO THE OBEX REGION IN RANID FROGS. T. J. Neary. Anatomy Department, Creighton University, Omaha, NE 68178.

Ranid frogs received unilateral 100nl injections of 15% HRP in the spinal cord or obex region. Following survival times of 4-5 days, the animals were perfused with buffered 2% gluteraldehyde. The brains were embedded in gelatin, cut at 40 microns, and the sections processed with tetramethyl benzidine. In cases where injections were confined to the region between the obex and the brachial enlargement, two forebrain areas were strongly labelled. The first area was the caudal portion of the dorsal and ventral striatum. In one transverse section, over 180 labelled neurons were counted in the striatum on the side ipsilateral to the injection site. A few labelled cells were also seen on the contralateral side in this case, but may have been present because of HRP spread. The other heavily labelled area was the rostral portion of the anterior preoptic area, although labelling here was considerably less dense than in the striatum. Most of the cells in the anterior preoptic area were on the side ipsilateral to the injection site. Other forebrain areas were also labelled, but to a much lesser extent. These were: the medial amygdala, magnocellular preoptic nucleus, suprachiasmatic nucleus, lateral and dorsal hypothalamic nuclei, posterior tuberculum, ventromedial thalamic nucleus, both parts of the ventrolateral thalamic nucleus, nucleus of Bellonci, and posterior thalamic nucleus. Nearly all of these populations were labelled primarily on the ipsilateral side. These results are similar to those reported by ten Donkelaar in Xenopus (Neurosci., 6:2297) following HRP injections of the brachial enlargement. He did not report the rostral extent of spread from his injections, however, and it remains to be determined whether the striatum and anterior preoptic area project to both the caudal medulla and spinal cord or only to the caudal medulla. Autoradiographic studies are now underway in order to answer this question.

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219.17 FINE STRUCTURE OF THE MAIN AND THE ACCESSORY OLFACTORY BULBS IN GARTER SNAKE (Thamnophis sirtalis). B.M. Binder* and D.L. Atkins* (SPON: R. Bohn). Dept. of Biological Sciences, The George Wash. Univ., Wash., D.C. 20052.

Ultrastructure of the main and the accessory olfactory bulbs was examined in garter snake (Thamnophis sirtalis). Comparison of the two bulb types showed few differences in neuron morphology, synaptic structure or profiles, or general organization.

Three neuron types were distinguished based on location, size, and organelle content. External granule cell perikarya were found in the glomerular, external plexiform, and mitral cell layers. They were round to oval with scant cytoplasm predominantly filled with ribosomes. Internal granule cell perikarya displayed similar fine structure to external granule cells, but were found in the internal plexiform and granule cell layers. Mitral cells were much larger than the other neuron types and contained large amounts of cytoplasm with extensive Golgi apparatus and Nissl substance.

Five synapse types were characterized based on neuronal processes involved, vesicular shape, and appearance of synaptic density. External and internal granule cell somata received synapses from axons of undetermined origin. Presynaptic vesicles were round and the synaptic density was asymmetric. Axon terminals with identical appearance to those synapsing on granule cells, synapsed on dendrites in all layers except the nerve and granule cell layers. Mitral cells engaged in reciprocal synapses with dendritic gemmules; reciprocal synapses between mitral cell dendrites and gemmules showed similar fine structure to reciprocal dendrosomatic synapses and to reciprocal dendrodendritic synapses described in mammals (Willey, T.J., J. Comp. Neurol., 152: 211-232, 1973). Axodendritic synapses from primary nerve axons occured exclusively in glomeruli.

Fine structure of the main and accessory olfactory bulbs in garter snake was similar, but not identical to that described in mammals.

219.18 A PRETECTAL-TECTAL ENKEPHALIN CONNECTION: IMMUNOHISTOCHEMICAL STUDIES OF HOMOLOGOUS SYSTEMS IN REPTILES. Steven E. Brauth & Anton Reiner. Dept. of Psychology, Univ. of Md., College Park, Md. & Dept. of Neurobiology & Behavior, SUNY, Stony Brook, N.Y.

The avian nucleus spiriformis lateralis (SpL) receives a prominent input from the ipsilateral basal ganglia and projects massively, and apparently exclusively, upon the tectum. The SpL projection is enkephalinergic and terminates within deeper tectal layers (8-13). A similarly situated pretectal cell group in reptiles, called the dorsal nucleus of the posterior commissure (nDCP), receives basal ganglia input and projects upon deep tectal layers in caiman, turtles and lizards (Reiner et al, JCN, '80; ten Donkelaar et al, Neurosci., '81). To further examine similarities between the avian SpL and the reptilian nDCP, we have used immunohistochemical methods to characterize the distribution of leucine enkephalin-like immunoreactivity in both the tectum and pretectum of turtles (Chrysemys scripta), caiman (Caiman crocodilus) and lizards (Sceloporus)(antibodies supplied by K.-J. Chang).

In all three species studied, many enkephalinergic neurons were observed within nDCP. In lizards this cell group has also been called the medial pretectal nucleus (Butler and Northcutt, JCN, '73). In addition, the cells of nDCP could be observed to give rise to enkephalinergic processes which could be traced into deep tectal layers. Numerous enkephalinergic fibers were observed within both deep and superficial tectal layers in all three species.

In both turtles and lizards, enkephalinergic neurons were observed within the deeper tectal layers. These neurons give rise to ascending enkephalinergic processes that ramify as fine fibers within superficial tectal layers. In caiman, fine enkephalinergic fibers were observed within the superficial tectal layers, but these fibers could not be traced to a source within deeper tectal layers. An additional system of coarse enkephalinergic fibers was present along the inner margin of the optic tract in all species studied. In lizards, numerous enkephalinergic cell bodies were seen in the optic tract layer; these neurons contribute fibers to the coarse fiber plexus along the inner margin of the optic tract.

The present results indicate that the reptilian nDCP is comparable to the avian SpL not only in terms of projections, but in terms of enkephalin content as well. In light of the enkephalinergic projection of SpL to deep tectal layers and in light of the results of anatomical studies showing that nDCP projects to the tectum in reptiles, we suggest that enkephalinergic fibers of the deep tectal layers in turtles, caiman and lizards probably arise from nDCP neurons. The present results indicate that enkephalin-mediated basal ganglia control over the tectum may be a ubiquitous aspect of the organization of the sauropsid nervous system.

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219.19 DORSAL COLUMN NUCLEI IN CROCODILIA. Michael B. Pritz. Division of Neurosurgery, University of California Irvine Medical Center, Orange, CA 92668.

Anatomical identification of a dorsal column nucleus was investigated in 2 species of Crocodilia by means of certain afferents. In 4 juvenile Caiman crocodilus dorsal rhizotomy proximal to the ganglion was performed in either forelimb or hindlimb regions of the spinal cord under cold narcosis. After survival periods of 15 to 24 days, animals were given a lethal overdose of intraperitoneal pentobarbital sodium and perfused transcardially with 10% formalin. Brains were processed according to standard techniques for the determination of anterograde degeneration. Degenerating axons resulting from dorsal rhizotomy in the hindlimb area could be traced into a medial portion of the dorsal funiculus to terminate in a discrete medial part of the medulla. Degenerating axons from dorsal rhizotomy in the forelimb area could be followed into a lateral part of this same nuclear group in the medulla. This area was termed the dorsal column nucleus (DCN). In 2 juvenile Alligator missipiensis, electrolytic lesions were stereotaxically placed in the dorsal funiculus under parenteral methohexital sodium and local anesthesia. Each brain was processed by similar anterograde degeneration techniques after survival periods of 25 to 28 days. Massive terminal degeneration was seen in a region of medulla identical to that found in Caiman. This region was felt to represent the DCN in Alligator.

These preliminary experiments served as a basis to examine the morphology of the DCN in these 2 species of Crocodilia. When stained with cresyl violet, positive and reliable identification of the DCN in transverse sections proved difficult. While fiber stained preparations better outlined this area, histochemical enzymatic activity of succinate dehydrogenase (SDH) and acetyl cholinesterase (AChE) proved to be the simplest and most reliable means to identify the DCN. The DCN was readily visualized in this fashion because it stained intensely with both SDH and AChE.

219.20 RETINAL GANGLION CELL DISTRIBUTION IN FERRET. Z. Henderson. Univ. Lab. of Physiol., Parks Rd., Oxford OX1 3PT, England.

Recently Linden et al., J. Comp. Neurol., 203: 189-211, 1982, showed that the ferret is a useful animal for developmental studies on carnivore visual system. I have made a study of retinal ganglion cells in Nissl-stained retinal whole mounts as a preliminary to experiments involving retrograde axonal transport. Retinae were obtained from female pigmented ferrets 0.6-0.9 kg, perfused with formol-Ringer. Ganglion cells were counted, and cross-sectional areas of cells were measured from sample points all over the retina. Retinal whole mounts from newborn ferrets were also examined.

In adults the density of cells ranges from 400/mm^2 at the superior edge of the retina to 5300/mm^2 in the central area. Although the central area is small, the density gradient between central and peripheral retina is not as steep as in the cat. The width of the retina across its horizontal extent is on average 10 mm and the optic disc lies 4 mm from the temporal edge and 6 mm from the nasal edge. The central area is 2 mm from the temporal edge of the retina. A long, narrow visual streak of high cell-density extends horizontally from the central area to midway between the optic disc and the nasal edge of the retina. There is a wider variety of cell shapes and sizes in any given area than in cat retina and there is no overall trend towards smaller cell sizes in high-density areas. Histograms of cell sizes peak around 50-90 μm^2 and have a long tail, up to 300-400 μm^2, in both high-density and low-density areas.

The retinal ganglion cell layer of newborn ferret is similiar to that of prenatal kitten at embryonic day 47 (Stone et al., Dev. Brain Res., 2: 321-242, 1981). There are two classes of cell, a darkly staining type presumed to be a glial cell and a pale-staining type , about 55 μm^2 in size, resembling a neurone. Both types are evenly distributed across the retina and there is no obvious difference in cell density between the middle and the edge of the retina. Hence the ferret provides an excellent opportunity for studying the development of regional specialisation in the retina.

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220.1 INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION BY CEREBRAL CORTICAL STIMULATION. S. Pretel, M. Fraunhoffer*, J.D. MacKinnon and E. Carstens. Dept. Animal Physiology, Univ. Calif., Davis, CA 95616

Cerebral cortical and pyramidal tract stimulation has excitatory and inhibitory effects on spinal dorsal horn neurons. However, there is little information regarding corticospinal modulation of nociceptive transmission. We therefore investigated whether the responses of dorsal horn neurons to controlled noxious skin heating could be affected by cerebral cortical stimulation, a possibility suggested by our previous observation that spinal neurons are powerfully inhibited by stimulation of the cerebral peduncles and internal capsule (Fraunhoffer et al., this volume).

The responses of single lumbar dorsal horn units to noxious radiant heat stimuli (50°C, 10 sec) applied to glabrous hindfoot skin were recorded with microelectrodes in cats anesthetized with sodium pentobarbital and N_2O. Intracortical stimulation (100 msec trains at 100 Hz, 3/sec) was delivered via a bipolar semi-microstimulating electrode positioned in the hindlimb region of the somatosensory cortex. The dorsal horn unit heat-evoked response during concomitant cortical stimulation was expressed as a % of the unit's control response without cortical stimulation.

For more than 30 units to date, the responses of most were markedly reduced (to 0-60% of control) during stimulation (50-150 µA) at sites histologically localized within the contralateral medial posterior sigmoid gyrus, subjacent white matter, and underlying upper bank of the cruciate sulcus. These sites correspond roughly to those from which cat spinocervical neurons were inhibited (Brown et al., J. Physiol. 264:1, 1977). Stimulation at corresponding sites in the ipsilateral cortex was ineffective. Unit responses to a series of graded noxious heat stimuli increased linearly from threshold (39-44°C) to 52°C. When the temperature series was repeated in 13 units during concomitant intracortical stimulation, the slopes of the temperature-response functions were reduced with small changes in the response threshold. A similar effect was also produced by stimulation of the ipsi- (7 units) or contralateral (2 units) cerebral peduncle or internal capsule. To study a possible role for 5-hydroxytryptamine (5-HT) in corticospinal inhibition, we tested whether this inhibition could be reduced by systemic administration of the 5-HT antagonist methysergide. Inhibition produced by stimulation of the contralateral cortex (5 units) or ipsilateral cerebral peduncle or internal capsule (4 units) was unaffected or slightly reduced following methysergide (0.5-1 mg/kg), indicating that 5-HT is not the primary transmitter mediating corticospinal inhibition. This suggests that the pathway for corticospinal inhibition may be primarily separate from serotonergic descending inhibitory pathways originating in the brainstem. We are currently investigating the role of direct corticospinal fibers in descending inhibition.

220.2 INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION BY LATERAL HYPOTHALAMIC STIMULATION. S.N. Suberg, M. Fraunhoffer* & E. Carstens. Dept. Animal Physiology, Univ. of Calif., Davis, CA 95616.

Electrical stimulation of the medial brainstem produces analgesia which is associated with powerful descending inhibition of spinal dorsal horn neuronal responses to noxious inputs. Analgesia also results from stimulation of the lateral hypothalamic (LH) area. To investigate whether this analgesia might also be mediated by descending spinal inhibition, we tested whether LH stimulation inhibited the responses of spinal dorsal horn neurons to controlled noxious radiant skin heating.

Tungsten microelectrodes were used to record the responses of single lumbar dorsal horn units to noxious radiant heat stimuli (50°C, 10 sec) applied to glabrous hindfoot skin in cats anesthetized with sodium pentobarbital and N_2O. The high-frequency heat-evoked response of each of 38 units was markedly reduced during concomitant bipolar electrical stimulation (100 msec trains at 100 Hz, 3/sec, 25-300 µA) at sites histologically localized to LH. Inhibitory sites in LH were systematically mapped by varying the depth of the stimulating electrode in parallel tracks spaced at 1-2 mm intervals; such medio-lateral maps were constructed at anterior levels +8 to +12. At each stimulation site, the magnitude of the unit heat-evoked response during LH stimulation was expressed as a % of the control response without LH stimulation. Powerful inhibition was generated from a continuous region extending from the periventricular gray laterally to the cerebral peduncles on either side, and ventrally to the base of the brain. The magnitude of inhibition increased with graded increases in LH stimulation intensity. For 14 units, the current strength at threshold for generating inhibition was about 30 µA for both ipsi- and contralateral LH sites. Responses of dorsal horn units to a series of graded noxious heat stimuli increased linearly from threshold (39-45°C) to 52°C. When the temperature series was repeated during concomitant ipsilateral LH stimulation, slopes of the temperature-response functions were reduced without significant changes in threshold. Similar results were obtained with contralateral LH stimulation. This effect is similar to that produced by medial hypothalamic and midbrain periaqueductal gray (PAG) stimulation.

A possible role for 5-hydroxytryptamine (5-HT) was tested by determining whether descending inhibition from LH could be blocked by acute systemic administration of the 5-HT antagonist methysergide (0.3-1 mg/kg). Inhibition was reduced in 8 of 9 units, and was unaffected in the remaining unit, following methysergide. This suggests that 5-HT may be partially involved in the mediation of descending inhibition from LH, perhaps via connections with 5-HT-containing neurons in the PAG or lower brainstem.

220.3 MAP OF DIENCEPHALIC SITES AT WHICH STIMULATION INHIBITS SPINAL NOCICEPTIVE TRANSMISSION. M. Fraunhoffer*, J.D. MacKinnon and E. Carstens (SPON: R.P. Scobey). Dept. Animal Physiology, Univ. Calif., Davis, CA 95616.

Electrical stimulation along the antero-posterior (AP) extent of the medial diencephalic periventricular gray (PVG) region powerfully inhibits the responses of spinal dorsal horn neurons to noxious skin heating (Carstens, this volume). To map the lateral extent of this inhibitory region, and to investigate the possible contribution of other diencephalic structures to descending modulation of spinal neurons, we systematically tested the effects of electrical stimulation at sites in a matrix spanning the AP and medio-lateral extent of the diencephalon on the responses of spinal dorsal horn neurons to controlled noxious skin heating.

The responses of single lumbar dorsal horn units to noxious radiant heat stimuli (50°C, 10 sec) applied to glabrous hindfoot skin were recorded with tungsten microelectrodes in cats anesthetized with sodium pentobarbital and N_2O. A comb of 5 parallel bipolar semi-microstimulating electrodes spaced at 2 mm intervals was stereotaxically lowered into the brain in 1-2 mm steps. At each site, the magnitude of the dorsal horn unit's response to heat during concomitant stimulation (100 msec trains at 100 Hz, 3/sec, 300 µA) was expressed as a % of the unit's preceeding control response without brain stimulation. Trials with and without brain stimulation alternated at 3 min intervals. In individual experiments, the comb of stimulating electrodes was positioned at one AP level (range: +6 to +16) either ipsi- or contralateral to the spinal recording site.

Throughout the AP extent of the diencephalon, unit heat-evoked responses were powerfully inhibited (to 0-50% of control) by stimulation of the thalamus and hypothalamic PVG near the midline. No inhibition was generated from the ipsilateral ventrobasal thalamic nuclei 2 mm or more lateral to the midline. Inhibition was generated from the contralateral ventrobasal thalamic nuclei. More powerful inhibition was generated from the subjacent lateral hypothalamic area, in a continuous region extending laterally from the PVG to the cerebral peduncles on both sides, and ventrally to the base of the brain. Powerful inhibition was also generated from the cerebral peduncles bilaterally. At more anterior levels (+13 to +16), inhibition was generated bilaterally from the internal capsule and from a large subjacent region encompassing the medial and lateral preoptic, septal (diagonal band), and more lateral regions. Results with capsular and peduncular stimulation suggest cortical involvement in descending inhibition.

Extensive diencephalic regions contribute to descending spinal inhibition. We are currently investigating whether these areas constitute one homogeneous system, or components in functionally separate inhibitory systems.

220.4 THE PEPTIDERGIC ORGANIZATION OF THE PERIAQUEDUCTAL GREY OF THE CAT: SUBSTANCE P AND VIP. Michele S. Moss and Allan I. Basbaum, Dept. of Anatomy, University of California, San Francisco 94143

The periaqueductal grey (PAG) is a component of an endogenous pain suppression system which has been implicated in stimulation produced and opiate-induced analgesia. In addition to the endorphins, other nonopioid peptides such as substance P (Sub P) and vasoactive intestinal polypeptide (VIP) produce potent analgesia when administered at central grey levels. Our previous studies of the distribution of enkephalin (ENK) immunoreactivity revealed that ENK-containing perikarya and terminals are located in discrete populations. As part of our analysis of the neuronal circuitry underlying the PAG's role in pain suppression, this study examined the distribution of Sub P and VIP immunoreactivity using PAP immunocytochemistry. Both untreated and colchicine treated cats (100ug/5ul,III ventricle) were used for this study. Caudal to the IV nerve nucleus, the densest population of Sub P-containing neurons is located in the lateral PAG. Unlike the distribution of ENK neurons only a few Sub P perikarya are seen in the caudal ventrolateral PAG and in the dorsal raphe nucleus. In the mid to rostral PAG, from the level of the IV nucleus to the level of the posterior commissure, a population of Sub P cells is still present in the lateral PAG. A second large population appears in the dorsal and dorsolateral PAG. Many Sub P-containing neurons are also found in the Edinger-Westphal nucleus and in the more rostral, periventricular grey. At the most caudal PAG levels, the Sub P terminal field staining is densest in the ventrolateral PAG. In the mid to rostral PAG, the densest Sub P terminal staining is found in the lateral PAG, adjacent to the aqueduct, and in the dorsal and dorsolateral PAG. Regardless of the rostral-caudal level examined, we found that VIP containing neurons are tightly clustered in the subependymal neuropil of the ventromedial PAG: the largest population is located at the levels of the III and IV nerve nuclei. We also found scattered VIP cells in the ventral PAG at these levels and in the dorsal raphe. Many VIP-labelled processes arborize within the subependymal neuropil. Terminal-field labelling, when present, is limited to the ventral and ventrolateral PAG.

In conclusion, Sub P and VIP neurons and terminals in the PAG, like those of ENK, are found in discrete populations. Their distribution demonstrates the heterogeneous nature of the PAG and indicates that the analgesic action of these peptides may operate via different neuronal circuitry.

220.5 QUANTITATIVE COMPARISON OF THE INHIBITION BY MORPHINE AND STIMULATION IN THE MESENCEPHALON ON NOXIOUS-EVOKED RESPONSES OF DORSAL HORN NEURONS IN THE CAT. M.Zimmermann, J.Sandkühler*, J.G.Thalhammer* and G.F.Gebhart. II.Physiol.Institut, Univ.Heidelberg, D-6900 Heidelberg, G.F.R.

Morphine (MOR) administration and focal electrical stimulation (Stim) in the periaqueductal gray (PAG) are antinociceptive and attenuate noxious-evoked neuronal responses in the spinal dorsal horn. It is unclear, however, whether MOR and Stim act at distinct and seperate sites in the PAG and/or via functionally seperate descending systems. Thus, the effects of Stim (bipolar coaxial electrodes, 100 Hz trains at 3/sec for 40 sec) and MOR (10-20 µg) applied at identical sites in the midbrain were examined quantitatively on spinal neuronal heat-evoked responses.
Lumbar dorsal horn neurons having both A- and C-fiber input from hindlimb cutaneous nerves were studied in anesthetized, paralyzed cats. Responses evoked by noxious radiant heat (50°C, 10 sec) applied to the cat's footpad were decreased to 12-100 % of control by MOR and to 1-72 % of control by Stim at identical mesencephalic sites. Both the maximal inhibition of heat-evoked neuronal discharges and the time to 1/2 maximal inhibition following MOR microinjection were correlated with the distance from the cerebral aqueduct: the closer MOR was administered to the aqueduct, the more rapid and efficacious was the inhibition. There was no correlation found between the distance from the aqueduct and the inhibition of spinal neuronal heat-evoked discharges by Stim. Stim sites both within and lateral to the PAG were efficacious.
Stimulus-response functions (SRF; neuronal discharge frequency versus stimulus temperature, 40-50°C) were linear. MOR administered in the PAG induced a change in the slope of the SRF without altering the neuronal response threshold; Stim in the PAG similarly affected the SRF. MOR administered i.v. (1 mg/kg) significantly attenuated spinal neuronal heat-evoked discharges and affected the slope of the SRF in the same manner as MOR administered in the PAG. The effects of MOR were antagonized by naloxone. A lesser dose of MOR (100 µg i.v.) did not affect the heat-evoked discharges. Thus, while the effects of MOR following its intra-PAG administration are mimicked by i.v. MOR (1 mg/kg), they are not due to the diffusion of MOR into the circulation. Access to tissue in or around the cerebral aqueduct, however, is necessary to MOR's efficacy in attenuating spinal nociceptive transmission. Stim, on the other hand, is effective at sites widespread in the mesencephalon (e.g., see Carstens et al., J.Neurophysiol. 43:332, 1980).

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220.6 QUANTITATIVE COMPARISON OF THE INHIBITION BY STIMULATION IN THE PAG AND NRM ON NOXIOUS-EVOKED RESPONSES OF DORSAL HORN NEURONS IN THE CAT. G.F.Gebhart, J.Sandkühler*, J.G.Thalhammer* and M.Zimmermann. II.Physiol.Institut, Univ.Heidelberg, D-6900 Heidelberg,GFR

Focal electrical stimulation (Stim) in the periaqueductal gray (PAG) and nucleus raphe magnus (NRM) is antinociceptive and significantly attenuates noxious-evoked neuronal responses in the spinal cord dorsal horn. There are few direct spinopetal fibers from the PAG and it is generally considered that the inhibition of spinal nociceptive transmission effected in the PAG occurs through the NRM, from which spinopetal fibers to the dorsal horn have been well documented (see Basbaum and Fields, Ann.Neurol. 4:451, 1978). The purpose of this study was to examine quantitatively the effects of Stim in the PAG and NRM on the noxious-evoked activity of the same dorsal horn neurons.
Lumbar dorsal horn neurons with A- and C-fiber input from hindlimb cutaneous nerves were studied in anesthetized, paralyzed cats. Comparison of the effects of Stim (bipolar coaxial electrodes, 100 Hz trains at 3/sec for 40 sec) in the PAG and NRM on the responses of the same dorsal horn neurons to radiant heat (50°C, 10 sec) applied to the cat's footpad indicated that Stim in the NRM was reliably more efficacious than Stim in the PAG at the same intensity of Stim current. Examination of the recruitment of inhibition (i.e., amount of inhibition with increasing currents of stimulation), however, revealed that Stim at either site produced the same increment in inhibition of spinal nociceptive transmission per 100 µA increase in Stim current; the threshold for inhibition was lower from the NRM than from the PAG, but the slopes of recruitment were the same. Discharges to A-fiber stimulation were reduced by NRM Stim at short delays (2 msec), but were unaffected by PAG Stim.
Stimulus-response functions (SRF; neuronal discharge frequency versus stimulus temperature, 40-50°C) were linear. PAG Stim changed the slope of the SRF without altering the neuronal response threshold. NRM Stim, however, produced a parallel shift in the SRF of 10 of 14 units. Thus, while Stim in the PAG affects the "gain control" of noxious heat intensity coding of dorsal horn neurons, Stim in the NRM appears to modulate the set point and threshold of noxious heat intensity coding without affecting its amplification (e.g., see Zimmermann, J.Physiol.Paris, 73:221, 1978). These and other data (see Sandkühler et al., this volume) suggest that the primary inhibitory pathway activated by Stim in the PAG does not descend through the NRM, but rather lateral to it.

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220.7 LIDOCAINE MICROINJECTED IN THE NRM DOES NOT BLOCK THE INHIBITION BY STIMULATION IN THE PAG OF NOXIOUS-EVOKED RESPONSES OF DORSAL HORN NEURONS IN THE CAT. J.Sandkühler*, J.G.Thalhammer*, G.F. Gebhart and M.Zimmermann. II.Physiol.Institut, Univ.Heidelberg, D-6900 Heidelberg, G.F.R. (SPON: ENA)

Focal electrical stimulation (Stim) throughout the mesencephalon and medulla inhibit noxious-evoked neuronal excitation in the spinal dorsal horn. At least two separate systems of descending inhibition can be activated in the mesencephalon (Carstens et al. J.Neurophysiol. 43:332, 1980): one in the periaqueductal gray (PAG) and the other lateral in the reticular formation (LRF). Inhibition of spinal nociceptive transmission can also be effected from the nucleus raphe magnus (NRM) as well as from the medullary reticular formation (MRF).
Lumbar dorsal horn neurons having both A- and C-fiber input from hindlimb cutaneous nerves were examined in anesthetized, paralyzed cats. The efficacy of Stim (bipolar coaxial electrodes, 100 Hz trains at 3/sec for 30 sec) to inhibit heat evoked (50°C, 10 sec) spinal neuronal responses was established for each of 4 Stim sites: PAG, LRF, NRM and MRF. The LRF placement was 4 mm lateral to the PAG site, the MRF placement 2.5 mm lateral to the NRM site. The inhibition by Stim in the MRF was examined quantitatively and found to be similar in all respects to the inhibition effected from the NRM (e.g., Stim in the MRF produced a parallel shift in the noxious heat intensity coding of dorsal horn neurons; see Gebhart et al., this volume).
After establishing the inhibitory efficacy of all 4 Stim sites, Lidocaine (Lido; 10 µg in 1 µl) was microinjected in the NRM or MRF at the site identical to Stim. The efficacy of Stim in the PAG and LRF was then examined when the NRM, MRF or both the NRM and MRF were reversibly blocked. When the efficacy of NRM Stim was blocked by Lido, the efficacy of the inhibition of spinal responses by Stim in the PAG or LRF was unaffected. When the MRF was blocked by Lido, however, the efficacy of inhibition from the PAG and LRF was significantly affected. The inhibition of spinal responses from the PAG and LRF was completely blocked only when Lido had been microinjected in both the NRM and MRF. The microinjection of Lido in the NRM and MRF had no effect on either the spontaneous activity of the neurons or their response to noxious heat, suggesting the absence of tonically active inhibitory systems under the experimental conditions. Thus, while the major descending inhibitory pathway from the mesencephalon appears to course laterally through the medulla (see also Gebhart et al., this volume), the NRM appears to also play a role.

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220.8 ELECTRICAL STIMULATION OF NUCLEUS RETICULARIS PARAGIGANTOCELLUARIS INHIBITS FELINE SPINAL CORD DORSAL HORN NOCICEPTIVE AND NONNOCICEPTIVE NEURONS. Bruce G. Gray and Jonathan O. Dostrovsky. Department of Physiology, University of Toronto, Toronto, Canada, M5S 1A8.

Many studies investigating the possible circuitry for descending inhibition of spinal cord dorsal horn neurons have proposed that the analgesic and inhibitory effects of stimulation of the periaqueductal gray are mediated by a relay through nucleus raphe magnus (NRM) which in turn inhibits the responses to nociceptive stimuli in the spinal cord. It has recently been suggested that a region lateral to NRM, and just medial to the facial nuleus, referred to as nucleus reticularis paragigantocellularis (PGL), may play a similiar role since it contains serotoninergic neurons which project to the spinal cord and has been shown to produce analgesia in the rat when stimulated. The aim of this study was to investigate the effects of electrical stimulation of PGL on the responses of nociceptive and nonnociceptive neurons.

Experiments were performed on chloralose anesthetized cats. Single unit recordings from lumbar spinal cord dorsal horn neurons were obtained using glass-coated platinum-plated tungsten microelectrodes. A bipolar stimulating electrode was stereotaxically implanted in the PGL and the stimulation site subsequently verified histologically. A total of 22 neurons was studied. These neurons were identified according to standard criteria as nociceptive (wide dynamic range and nociceptive specific) and nonnociceptive (low threshold mechanoreceptive) by a careful sensory examination. Some of these neurons were also shown to project to the lateral cervical nucleus. The inhibitory effects produced by PGL stimulation were tested on the responses of neurons excited at just suprathreshold levels by electrical skin stimulation. The brainstem conditioning stimulus consisted of a 100 ms 500 Hz train of 0.1 ms pulses delivered 130 ms prior to the peripheral stimulus. Stimulation of PGL inhibited 93% of the nociceptive and 90% of the nonnociceptive neurons with mean currents of 65uA and 67uA respectively. The effects were found to be similar for cells projecting to the lateral cervical nucleus. A comparison of these results with those previously obtained for stimulation of NRM revealed that while both regions inhibited over 85% of the nociceptive and nonnociceptive neurons, the mean current thresholds were lower for stimulation in PGL.

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220.9 SEROTONIN INVOLVEMENT IN DESCENDING INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION BY MEDIAL DIENCEPHALIC AND SEPTAL STIMULATION. M.J. Guinan*, J.D. MacKinnon and E. Carstens (SPON: E. Sassenrath). Dept. Animal Physiology, Univ. Calif., Davis, CA 95616

Electrical stimulation along a continuous region spanning the medial diencephalic periventricular gray (PVG), preoptic, and ventromedial septal areas, powerfully inhibits the responses of spinal dorsal horn neurons to noxious skin heating (Carstens, this volume). Such inhibition may underly analgesia produced by stimulation of these areas. To investigate a possible role for 5-hydroxytryptamine (5-HT, serotonin) in descending inhibition from these areas, we tested whether this inhibition could be reduced or blocked by (1) acute administration of the 5-HT antagonist methysergide, and (2) depletion of central 5-HT levels with the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA).

Microelectrodes were used to record the responses of single lumbar dorsal horn units to controlled noxious radiant heat stimuli (50°C, 10 sec) applied to glabrous hindfoot skin in cats anesthetized with sodium pentobarbital and N_2O. Separate bipolar stimulating electrodes were stereotaxically positioned in the PVG and medial preoptic or septal area. Unit responses to heat during concomitant electrical stimulation (100 msec trains at 100 Hz, 3/sec, 25-600 μA) at each site were expressed as a % of the unit's control response without brain stimulation. Experiments were performed using previously untreated cats receiving methysergide (0.2-1 mg/kg i.v.), and cats pretreated 72 hr earlier with 500 mg/kg PCPA to test effects of 5-HT depletion.

In untreated cats, PVG stimulation inhibited (to 10-69% of control) unit heat-evoked responses. This inhibition was abolished in 6 of 14 units following methysergide, and was reduced in the remainder. Inhibition produced by medial preoptic and septal stimulation was also markedly reduced or abolished in each of 8 units following methysergide. In PCPA pretreated cats, 15 of 26 units were inhibited to varying degrees, while the remainder were not inhibited, by PVG stimulation at strengths up to 600 μA. The mean PVG current strength at threshold for inhibition was significantly higher in PCPA pretreated (145 ± 128 S.D. μA) compared to untreated cats (67 ± 67 μA), and mean inhibition at 300 μA was significantly weaker. Similarly, mean threshold for inhibition from the medial preoptic and septal areas was significantly higher in PCPA (98 ± 87 μA) compared to untreated cats (25 ± 19 μA), and inhibition at 100-300 μA was significantly weaker.

Disruption of the central action of 5-HT reduced or blocked descending inhibition from medial diencephalic and septal sites, indicating that 5-HT is involved in its mediation. Pathways for descending inhibition from these areas may include connections with 5-HT-containing neurons in the midbrain or medullary raphe nuclei.

220.10 INTRATHECAL METHYSERGIDE AND PHENTOLAMINE ANTAGONIZE STIMULATION-PRODUCED ANALGESIA FROM THE NUCLEUS RAPHE MAGNUS. D.L. Hammond and T.L. Yaksh*, Mayo Foundation, Rochester, MN 55905.

The purpose of this study was to evaluate the postulate that stimulation-produced analgesia is mediated by activation of monoaminergic neurons of the brainstem which project to the spinal cord. Male Sprague-Dawley rats were chronically implanted with an intrathecal catheter and a stimulating electrode stereotaxically positioned in the nucleus raphe magnus (NRM) of the medulla. Seven days later, baseline tail flick latency (TFL) was measured. The NRM was then stimulated for 15 sec (monopolar) at 25 or 50 Hz with 0.5 msec square wave pulses of 50 to 150 μA intensity and TFL was redetermined during stimulation. Those rats in which stimulation of the NRM produced at least a 100% increase in TFL over baseline latencies were allowed 10 min for recovery of TFL to baseline values. After this time, either the serotonergic antagonist methysergide (48 nmoles), the noradrenergic antagonist phentolamine (48 nmoles), or pH-matched saline was injected intrathecally in a 15 μl volume. Fifteen min later, the TFL was redetermined to assess whether these agents alone had altered baseline nociceptive threshold. The NRM was then stimulated again using the same current parameters and the TFL was measured. The effects of the two remaining drugs were similarly tested at intervals of no less than 5 days; the order of administration was randomized. Care was taken to employ the same current parameters on all days of testing. At the completion of the study, the rats were killed and the site of stimulation was located histologically. Stimulation of the NRM in 7 rats produced a significant elevation of TFL (8.5 ± 0.3 sec) as compared to baseline latencies (4.0 ± 0.1 sec). Intrathecal administration of saline vehicle did not attenuate this analgesia. In contrast, intrathecal administration of either methysergide or of phentolamine alone significantly attenuated the analgesia, reducing TFL to 6.4 ± 1.0 and 5.7 ± 1.0 sec, respectively. However, neither of these antagonists produced a complete blockade of the analgesia since TFL's were still elevated as compared to prestimulation baseline latencies. These data support the postulate that the analgesia produced by activation of the NRM is mediated by activation of serotonergic neurons which project to the spinal cord. In addition, activation of a bulbospinal noradrenergic pathway may also mediate this analgesia. The inability of either monoaminergic antagonist to completely antagonize the elevation of TFL suggests further that these monoaminergic systems may have been co-activated in parallel. (Supported by NS 16541 to TLY and NS 06538 to DLH, and by the Mayo Foundation.)

220.11 EVIDENCE FOR MODULATION OF NOCICEPTIVE THRESHOLD BY ALPHA ADRENERGIC RECEPTOR SUBTYPES IN THE NUCLEUS RAPHE MAGNUS. J. Sagen and H. K. Proudfit. Dept. Pharmacol., Univ. Ill. Coll. Med., Chicago, IL 60612.

Activation of neurons in nucleus raphe magnus (NRM) produces hypoalgesia which most likely results from inhibition of spinal cord pain transmission pathways. Previous reports from this laboratory suggest that noradrenergic (NA) neurons modulate the activity of NRM neurons. NA projections appear to be inhibitory since iontophoretically applied norepinephrine (NE) inhibits the activity of NRM neurons. Furthermore, blockade of NA receptors in the NRM by the microinjection of α-adrenergic antagonists produces potent analgesia. Thus, the NA input to the NRM appears to increase pain sensitivity by tonically inhibiting NRM neurons.

Pharmacological and physiological studies have differentiated α-adrenergic receptors into α_1 and α_2 subtypes. In the peripheral nervous system the presynaptic receptors involved in the feedback inhibition of NE release have been designated α_2 receptors, while postsynaptic receptors found on effector organs are designated α_1 receptors. In the central nervous system, binding studies have revealed the existence of two similar subclasses of α-adrenergic receptors. However, both of these sites appear to be postsynaptic. The present study investigated the nature of α-adrenergic receptor subtypes in the NRM and their role in the modulation of pain sensitivity.

Female Sprague-Dawley derived rats (250-300 g) were implanted with a microinjection cannula aimed at the NRM. Following recovery from surgery, baseline tail flick latencies were determined and one of the following drugs was injected into the NRM: α_1 agonist phenylephrine (5 or 10 μg); α_2 agonist clonidine (7.5, 15, or 30 μg); α_1 antagonist prazosin (2.5 or 5 μg); α_2 antagonist yohimbine (1.5 or 3 μg); or vehicle. Tail flick latencies were assessed at 10, 20, and 30 minutes following the injection. Results were consistent with the classical model of postsynaptic α_1 receptors and presynaptic α_2 receptors modulating NE release. Both the α_1 antagonist prazosin and the α_2 agonist clonidine produced a dose-dependent increase in nociceptive threshold. This is expected since blockade of the inhibitory NA input to the NRM either by directly blocking the postsynaptic receptor or by presynaptic inhibition of NE release should result in hypoalgesia. Conversely, both the α_1 agonist phenylephrine and the α_2 antagonist yohimbine produced a dose-dependent decrease in nociceptive threshold. Since NE is inhibitory, NRM neurons would be inhibited either directly by combination of the α_1 agonist with the postsynaptic receptor or indirectly by the increased release of NE induced by inhibition of the presynaptic receptor. Thus, in the region of the NRM, both presynaptic α_2 and postsynaptic α_1 receptors may be involved in the modulation of pain perception. (Supported by USPHS Grant NS 12649 and the PMA Foundation)

220.12 EVIDENCE FOR THE MODULATION OF NOCICEPTIVE THRESHOLD BY ALPHA TYPE 2 ADRENERGIC RECEPTORS IN THE RAT SPINAL CORD. H. K. Proudfit and J. Sagen. Dept. Pharmacol., Univ. Ill. Coll. Med., Chicago, IL 60612.

Several lines of evidence suggest that bulbospinal noradrenergic (NA) neurons modulate nociceptive transmission in the spinal cord. For example, the direct application of α-adrenergic agonists into the spinal cord subarachnoid space produces analgesia (Reddy et al., JPET, 213:525, 1981). Conversely, the intrathecal administration of the α-adrenergic antagonist phentolamine increases sensitivity to painful stimuli (Proudfit and Hammond, Br. Res., 218:393, 1981). Thus, bulbospinal NA neurons appear to tonically inhibit spinal cord pain transmission pathways via α-adrenergic receptors.

In the peripheral nervous system, pharmacological differences exist between NA antagonists acting at post-synaptic receptors (α_1) and those acting on receptors located on NA terminals (α_2) which modulate NE release. The existence of similar subclasses of α-adrenergic receptors in the CNS is unclear. In these studies we sought to identify the -receptor subtypes involved in the modulation of nociceptive transmission in the spinal cord.

Male Sprague-Dawley derived rats (400-425 g) were implanted with an intrathecal catheter (PE 10 tubing) extending to the lumbar enlargement. Following recovery from surgery, baseline tail flick latencies were determined and each animal received an intrathecal injection of one of the following: phentolamine (7.5, 15, 30, or 60 μg); yohimbine (7.5, 15, or 30 μg); WB-4101 (15, 30, or 60 μg); DHE-45 (15, 30, or 60 μg); prazosin (30, 60, or 120 μg); or vehicle. Drugs were injected in a 15 μl volume followed by 10 μl of saline. Nociceptive threshold was again assessed 5, 15, and 30 minutes following the injection. Each of the drugs produced a dose dependent decrease in tail flick latency (hyperalgesia) within 5 minutes after the intrathecal injection. The order of potency was: yohimbine > phentolamine = DHE-45 > WB-4101 > prazosin. The potency of these compounds in producing hyperalgesia is correlated with their relative affinities for the α_2 receptor. Thus, NE tonically released from bulbospinal terminals may interact preferentially with receptors of the α_2 type. This is consistent with the autoradiographic studies by Young and Kuhar (PNAS, 77: 1696, 1980) showing that α_2 receptors are found in much higher numbers than α_1 receptors in the substantia gelatinosa of the spinal cord. Thus, primarily α_2-adrenergic receptors appear to be involved in the tonic modulation of nociceptive transmission in the spinal cord. (Supported by USPHS Grant NS 12649 and the PMA Foundation)

220.13 THE INDUCTION OF ANALGESIA BY THE LOCAL INJECTION OF CARBACHOL INTO THE NUCLEUS RAPHE MAGNUS. Mark S. Brodie and Herbert K. Proudfit. Dept. Pharmacol., Univ. Ill. Coll. Med., Chicago, IL 60612.

Investigations in this and other laboratories have indicated that neurons located in the nucleus raphe magnus (NRM) are involved in the descending control of nociception. However, the control of these descending neurons by higher centers has not yet been elucidated. There is anatomical evidence for cholinergic terminals in the region of the NRM (Palkovits, M. and Jacobowitz, D.M.: J. Comp. Neurol. 157:29, 1974) which suggests that the the activity of NRM neurons may be modulated by cholinergic neurons. The present study was conducted to determine whether the cholinergic innervation of the raphe region is involved in modulating the activity of those raphe neurons which participate in regulating pain sensitivity. These studies involved microinjecting carbachol, a cholinergic agonist, into the NRM and measuring the resulting changes in pain sensitivity.

Sprague-Dawley derived rats (200-300 gm) were implanted with microinjection guide cannulae directed toward the NRM. One week later, microinjections were made, and nociceptive thresholds were measured using tail flick (TF) and hot plate (HP) tests. In the first series of experiments, carbachol was injected into the NRM at doses of 1.0, 2.5, and 5.0 µg in 0.5 µl saline. These injections produced dose-dependent hypoalgesia. The hypoalgesia resulting from 1.0 µg lasted about 60 min, while that produced by 5.0 µg was still evident 120 min after injection. The analgesia produced by carbachol injected into the NRM appears to be mediated by muscarinic receptors since it was reversed by atropine sulfate (2.0 mg/kg, s.c.).

To avoid possible confounding effects produced by the systemic injection of atropine, a second study was done microinjecting both carbachol and atropine into the NRM. Microinjection of carbachol (2.5 µg) into the NRM produced a potent hypoalgesia which was reversed by the subsequent microinjection of atropine sulfate (5 µg) at the same site. Carbachol-induced analgesia was not affected by subsequent microinjection of saline (0.25 µl). When atropine alone was microinjected in the NRM, it had no effect on nociceptive threshold, but did prevent the development of hypoalgesia by a subsequent microinjection of carbachol at the same site.

These data suggest that a cholinergic system activates raphe-spinal NRM neurons which decrease the perception of painful stimuli. The failure of atropine to alter pain sensitivity indicates that the cholinergic system is not tonically active. (Supported by USPHS Grant NS 12649).

220.14 SENSITIZATION OF NOCICEPTION DURING WITHDRAWAL FROM NARCOTICS CAN BE REVERSED BY 5-HYDROXYTRYPTOPHAN (5-HTP). R. Emmers. Dept. of Physiol., Coll. of P&S, Columbia U., New York, N.Y. 10032.

A recent study (Physiologist 24:25, 1981) has indicated that rats addicted to narcotics (morphine, meperidine) exhibit heightened sensitivity to mechanical stimulation. The sensitivity may increase to the point that innocuous mechanical stimuli elicit squeaks. Since the action of narcotics involves the nuclei raphe magnus which contain serotonergic neurons, a question was raised about the possible role of serotonin in such a sensitization. Acute electrophysiological experiments were performed using rats addicted to narcotics according to the addiction schedule of Kerr and Pozuelo (Mayo Clin. Proc. 46:653). Electrophysiological procedures for the evaluation of nociception were used (Emmers, R. PAIN: A spike-interval coded message in the brain. Raven Press, New York, N.Y. 1981). Responses were evoked in individual nociceptive neurons of the nucleus VPL of the thalamus by applying single electrical pulses to the contralateral sciatic nerve of rats anaesthetized with a mixture of chloralose and urethane. Spike potentials occurring within the initial 500 msecs following each stimulus applied at 2 sec intervals were deposited in a digital computer for gathering post-stimulus time histograms. The latter contained a burst of short-latency spikes followed by a lack of activity over 130-140 msecs and 2-3 late activity peaks distributed at 70-90 msec intervals. As indicated by experiments of the above cited reference, occurrence of the late spikes at specific intervals is a necessary condition for the arousal of pain via the spinothalamic system. The threshold stimulus for the computer accumulation of a single late spike interval was 6V (0.5 msec) in non-addicted animals. In the addicted rats, the threshold decreased to 2.5V during naloxone precipitated withdrawal. This stimulus is much below the intensity required for activation of the C-fibers (4V) in the rat sciatic nerve (see ref. above). Intracarotid infusion of 5-HTP (2.5 mg/kg) promptly increased the threshold for the recording of the late spike interval to 6V. Moreover, an infusion of 4 mg/kg morphine sulfate, that failed to erase the late spike intervals in addicted rats, became effective if the animal was infused also with 5-HTP. In behavioral experiments, innocuous taps applied to the hind-leg elicited squeaks during morphine withdrawal in addicted rats but required noxious stimulation after the infusion of 5-HTP. (Aided by grant DA-02916 from ADAMHA).

220.15 NALOXONE ANTAGONISES THE RAPHE MAGNUS-INDUCED INHIBITION OF ANTIDROMICALLY ACTIVATED ACTION POTENTIALS IN IDENTIFIED SPINOTHALAMIC TRACT CELLS. J.A. Pearson and P. Pinkhasik*. Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

The behavioural analgesia caused by stimulation of NRM can be antagonised by naloxone, and Rivot et al. have shown that naloxone reverses the NRM-induced inhibition of responses of dorsal horn neurones to noxious stimulation in the rat (Brain Res., 176, 355-364, 1979). In a recent study, Ruda has demonstrated the existence of enkephalin-containing synaptic terminals on the soma of identified spinothalamic tract neurones (Science, 215, 1523-1525, 1982). In view of the evidence for the involvement of opioid mechanisms in the control of pain transmission at the spinal level, and the importance of the NRM in the generation of this analgesia, the naloxone-sensitivity of NRM-dependent inhibition of spinal neurones was investigated in urethane anaesthetised rats.

Two populations of dorsal horn neurones were studied: a) spinothalamic tract (STT) neurones, identified by antidromic activation of axons ascending in the contralateral medial lemniscus, and b) unidentified neurones, which were sensitive to hair movement and touch, which also responded with short latency (7-11 msec) to weak transcutaneous electrical stimulation (<2mA, 0.2 msec). Data are presented from cells which were inhibited by stimulation of NRM. This stimulus consisted of a train of pulses (300Hz, 0.2msec, 45msec duration) which started 50 msec prior to stimulation of STT axons or the skin. Inhibition of responses of unidentified dorsal horn neurones, to non-noxious cutaneous stimulation could be achieved with significantly ($p<0.005$) less intense stimulation of the NRM (mean, 55.6 ± SE 6.2µA) than that required for inhibition of the antidromically evoked action potentials in identified STT cells (242.1 ± SE 82.7 µA).

Intravenous injection of naloxone (0.4 mg/Kg) resulted in a marked reduction of the NRM-driven inhibition of the antidromic action potentials in 60% of the STT cells, and of the orthodromically evoked responses in 50% of the unidentified neurones. The maximal effect of naloxone occurred between 6 and 12 mins after the injection, and the influence persisted for a period which often exceeded 1 hr.

The fact that the inhibition of antidromically evoked action potentials in STT neurones is sensitive to naloxone, suggests the involvement of opioid-mediated, post-synaptic inhibition of these cells following stimulation of NRM. This finding is clearly in accordance with Ruda's demonstration of enkephalin-containing synaptic terminals on the soma of STT neurones.

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220.16 MORPHINE ON TONIC SUPRASPINAL INHIBITION OF CAT SPINAL CORD NOCICEPTOR-DRIVEN NEURONES (NDN). Peter J. Soja* & John G. Sinclair, Division of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

Claims have been made that opiates produce analgesia partly by increasing inhibitory neurotransmission via (a) bulbospinal pathway(s). For example, Hanoaka et al. (J. Pharmacol. Exp. Ther.207: 476-484, 1978) reported that i.v. morphine was more effective in suppressing NDN in intact rather than spinal cord sectioned animals. However Duggan et al. (Br. J. Pharmacol. 69: 461-466,1980), utilizing the cold block technique, concluded that i.v. morphine decreases tonic bulbospinal inhibition of NDN. Transecting or cold blocking the spinal cord releases the dorsal horn NDN from a powerful tonic inhibitory impingement which is reflected by a tremendous increase in NDN response to noxious stimuli. Consequently, the interpretation of the above experiments is difficult because the antinociceptive effects of morphine may be greatly altered by the change in excitability of NDN after blocking the descending inhibition. Therefore the present experiments were initiated to compare the suppressive effects of morphine sulfate (1.0 mg/kg i.v.) with those of iontophoretically applied Y-aminobutyric acid (GABA, 0.5M, pH 3.6) on L_7 dorsal horn NDN before and after blockade of tonic supraspinal inhibition. In chloralose-anaesthesized cats extracellular unit activity was recorded from NDN which were activated by noxious radiant heat (47-59°C, 10-15 s, 2 min intervals, with feedback control). The degree of tonic inhibition, as judged by comparing the noxious heat-evoked response in the normal vs. cold block state (thermo-electric cooling device positioned at L_1), was determined for each neurone. With the cord in the normal state a slow infusion of morphine or iontophoretically applied GABA markedly reduced the number of noxious heat-evoked spikes/25 s from NDN (229.4 ± 41.0 to 67.9 ± 30.2, mean + S.E.M., n=7 and 175.1 ± 28.8 to 86.5 ± 15.0, n=12, respectively). With the spinal cord in the cold block state morphine and GABA similarly reduced the noxious heat-evoked responses of NDN (786.7 ± 112.4 to 391.5 ± 97.7, n=7 and 833.1 ± 168.9 to 498.1 ± 95.2, n=12, respectively). Morphine was tested on only one cell per animal. Interestingly our morphine results agree with those of Hanoaka et al. (1978) as well as those of Duggan et al. (1980) using their methods of calculation and interpretation.However, due to the marked similarity in action of locally applied GABA and systemically applied morphine, the results strongly suggest that morphine produces antinociception mainly through an action at the spinal cord level.

(Supported by the Medical Research Council of Canada).

220.17 ANATOMICAL EVIDENCE THAT SUPRASPINAL EFFERENTS TO THE SPINAL CORD MEDIATE VAGINAL STIMULATION-INDUCED ANTINOCICEPTION IN RATS. L.R. Watkins, Dept. Physiol., Med Coll. Virginia, Richmond, 23298, P.L. Faris*, Inst. Animal Behavior, Rutgers Univ., Newark, NJ,07102, D.J.Mayer(MCV),and B.R.Komisaruk(IAB) (SPON:B.Natelson).

Previous studies (Crowley, Rodriguez-Sierra, and Komisaruk, 1977: Brain Res. 137, 67; Steinman, Komisaruk, Yaksh, and Tyce, 1980: Proc. Soc. Neurosci. 6, 454) indicate that vaginal stimulation activates a supraspinal descending monoaminergic system that suppresses responses of rats to noxious stimulation. The present study analyzes the neuroanatomical basis of this system. Decerebration at the level of the colliculi did not significantly affect vaginal stimulation-produced antinociception (VSPA) (mean latency of tail flick to radiant heat in sec (TFL): sham control: 7.92; decerebrate: 7.88). However, complete spinalization at the 2nd thoracic vertebral level significantly reduced VSPA (TFL sham control: 7.93; T2 spinal transected: 5.17). These findings support earlier indirect evidence that neural pathways involved in VSPA originate in the lower brainstem and descend through the spinal cord. Since the dorsolateral funiculus (DLF) is known to be involved in other analgesia systems that are activated by supraspinal mechanisms (Murfin, Bennett, and Mayer, 1976: Proc. Soc. Neurosci. 2, 946; Basbaum and Fields, 1978: Ann. Neurol. 4, 451) we then determined the effect of DLF lesions on VSPA. Bilateral lesions of DLF at the T2 level of the spinal cord significantly reduced VSPA (TFL sham control: 7.95; DLF lesioned: 5.41) as effectively as complete spinalization. Thus, vaginal stimulation apparently activates higher order neurons in the lower brainstem, the projections of which descend in the DLF and inhibit pain transmission at the spinal level. These analgesia systems may be activated during mating and parturition in the rat. Supported by PHS Grant DA00576 (DJM) and NSF BNS78-24504 (BRK).

220.18 STIMULATION-INDUCED HYPERALGESIA. R.Dowman*and J.P. Rosenfeld. Cresap Neuroscience Lab.,Northwestern Univ., Evanston Il.,60201.

It is well known that prolonged exposure to noxious peripheral stimulation produces analgesia in rats (stress-induced analgesia). The present study was undertaken to determine if analgesia can be elicited by sub-noxious stimulation of a central pathway containing fibers responsive to noxious stimuli. Analgesia was measured immediately after each stimulation session using a facial heating device which has been verified in our laboratory several times. Stimulation consisted of a 200 us biphasic square wave delivered to the descending trigeminal tract (TT) at a frequency of 0.5 Hz. A group of 6 rats showed hyperalgesia ($p<.05$) on the ipsilateral (to the stimulating electrode) side of the face following 500 stimulations at levels that were 50% and 80% of a level previously determined to just evoke a noxious response. Hyperalgesia on the contralateral side of the face was seen only after stimulation at the 80% level ($p=.05$). The animals' nociceptive sensitivity after 150 stimulations at the noxious threshold (100% level) was found to be the same as baseline ($p>.10$). These results demonstrate that sub-noxious stimulation of the TT produces hyperalgesia. We are presently recording cortical somatosensory evoked potentials (SEP) during sub-noxious stimulation to determine if the observed changes in the nociceptive sensitivity can be correlated with central neural activity. Preliminary data indicates that the SEP is not altered during the course of stimulation. Likewise we have not found naloxone (1 mg/kg) to have any effect on the SEP. We will also be stimulating the animals at supra-threshold levels to determine if analgesia can be produced. (Supported by NIH grants GM23696 and DE05204).

220.19 CHANGES IN ACID PHOSPHATASE ACTIVITY IN THE SUBSTANTIA GELATINOSA OF 2- AND 15-DAY-OLD RATS IN RESPONSE TO PAIN. R. M. Kantner* and M. L. Kirby (SPON: P. Dyken). Dept. of Anatomy, Medical College of Georgia, Augusta GA 30912.

It has been postulated that acid phosphatase (AP) activity is involved in some aspect of neurotransmitter synthesis and/or degradation mediating nociceptive input in the dorsal horn and substantia gelatinosa (SG) specifically. After 6 days postnatally, AP activity is extremely dense in the SG, and fluctuations are difficult to detect. In at least two instances a reduced or lower level of AP has been demonstrated in the rat SG. First, in rats pretreated with capsaicin, AP levels in the SG have been greatly depleted. Secondly, prior to postnatal day 6, the normally developing rat has a low level of AP activity in the SG when compared to rats after 6 days postnatally. These two examples provided animal models by which the effects of a chronic-type chemogenic pain stimulus on AP activity in the SG were studied. Wistar rats were injected with either 50 mg/kg capsaicin in 10% alcohol, 10% Tween in .9% saline solution or vehicle on day 16 and 17 of gestation. Two-day and 15-day-old offspring were given subcutaneous injections of a 5% formalin solution or .9% saline to the dorsal aspect of the right forepaw. Four treatment groups were formed for each age group: formalin/control, formalin/capsaicin, saline/control, and saline/capsaicin. One hour after injection, animals were decapitated. Frozen sections were analyzed for acid phosphatase activity. Saline/capsaicin cervical and lumbar sections of 15-day-old rats had the expected minimal AP reaction in SG. Two-day-old saline/control animals demonstrated the expected low level AP activity in the SG for cervical and lumbar sections. Microdensitometric readings (1.0 mm aperture) indicated that the AP activity of the SG of formalin/capsaicin cervical sections was significantly greater than saline/capsaicin 15-day-old animals for right and left sides. In two-day-old animals microdensitometry showed that formalin/control right cervical sections had significantly higher AP activity than saline/control. Our results indicate a direct role for AP in pain transmission in the spinal cord. Measurements of increased AP activity were at a time when the rats were still feeling pain. The increase in AP activity in cervical SG was bilateral in 15-day-old formalin-injected animal (formalin/capsaicin) but ipsilateral (right side) in the 2-day-old formalin-injected animal. This difference may indicate contralateral projections of primary afferents or SG neuronal axons are not present in the 2-day-old rat, but are functionally complete in the 15-day-old rat. Supported by NIDA 02060.

220.20 Effects of limb position on pain perception in human subjects. J.A. McMillan and A.M. Moudy*. Biology Department, Montana State University, Bozeman, MT 59717

Many parallels between flexor reflex mechanisms and pain perception have been described, suggesting that the two functions may utilize similar, if not common, integrative substrates in the CNS. If common neuronal pools are shared, it might prove possible to enhance and depress nociception by voluntary activation and inhibition respectively of the flexor neuron pool. This report describes such changes in pain perception.

Experiments were performed on 14 naive subjects. Square-wave electrical shocks (10 ms) were administered to the skin of the 2nd toe of the right foot. Stimulus intensity was increased until verbal reports confirmed perception of both first and second pain. Series of 20-25 identical shocks were then given at 1 Hz. The right leg was held in one position (relaxed, extended or flexed) for the first 10 shocks and the subject was then instructed to move the leg to and hold it in another position until the trail of shocks ended. The subject was then asked to compare the pain while in the 2nd position relative to that in the first. Pooled results are given in the table below, which shows the number of times the different changes in perception were reported for each series of positions (R=relax, E=extend, F=flex).

	RE	RF	ER	EF	FE	FR
INCREASE	1	6	21	13	5	21
DECREASE	30	21	6	15	21	8
NO CHANGE	7	11	8	12	12	9

These results confirm unpublished observations that nociception is greatest when the limb is relaxed and that nociception is suppressed with displacement in either direction. However, they also show that pain perceived during flexion is stronger than during extension. When E followed R (RE), pain increased in only 3% of trials, whereas it increased in 15% of trials when F followed R ($P<.05$). Likewise, pain increased in 33% of trials when going from E to F, but only increased in 13% of trials when going from F to E ($P<.05$). Furthermore, the sequence of the 6 permutations in which there is a progressive increase in the proportion of times that pain during the second position was stronger than during the first (RE, FE, RF, EF, FR, ER) is the same as would be predicted on the assumption that pain will increase progressively from E to F to R ($P<.001$).

Based on these observations, we conclude that nociception does share some common integrative substrates with flexor function to the extent that descending modulation of flexor activity affects nociception in a parallel manner.

(Supported in part by NSF Grant ISP-8011449)

221.1 CHOLINE ADMINISTRATION ELEVATES BRAIN PHOSPHORYLCHOLINE CONCENTRATIONS. W. R. Millington and R. J. Wurtman. Laboratory of Neuroendocrine Regulation, M.I.T., Cambridge, Mass. 02139.

The phosphorylcholine (PCh) concentration of rat brain rises and falls in response to parallel changes in the availability of circulating choline. We found that the administration of a single oral dose of choline chloride (20mmoles/kg) elevated whole brain concentrations of choline (from 18.5 ± 1.5 to 44.8 ± 4.2 nmoles/g ± SEM) and PCh (from 228.2 ± 30.1 to 340.6 ± 18.3 nmoles/g); a greater proportion of exogenously administered choline was thus retained by the brain in its phosphorylated form than as the free amine. Striatal PCh concentrations were elevated within two hours of choline administration and continued to be significantly greater than control values up to thirty-four hours after treatment. The response of striatal choline levels to exogenous choline was of shorter duration (fourteen hours) than that of PCh and was correlated with a significant increase in striatal acetylcholine concentrations. The consumption of a choline free diet for seven days lowered both serum choline (from 13.0 ± 0.3 to 8.4 ± 1.5uM) and striatal PCh (from 689.0 ± 67.5 to 527.6 ± 39.2 nmoles/g) but had no effect on striatal choline or acetylcholine levels (control animals consumed diets containing 0.18% choline). These results suggest that choline kinase (ATP:choline phosphotransferase) is unsaturated by its substrate in vivo and may thus serve to modulate the response of brain choline concentrations to alterations in the supply of circulating choline.

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221.2 DIFFERENTIAL EFFECTS OF SOMAN AND DIISOPROPYLFLUOROPHOSPHATE ON LEVELS OF ACETYLCHOLINE AND CHOLINE IN RAT BRAIN AREAS. Tsung-Ming A. Shih. U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

In an effort to determine whether or not the central cholinergic effects produced by organophosphorus anticholinesterase compounds are of a universal nature, a comparison was made between acute soman and diisopropylfluorophosphate (DFP)-induced changes in levels of acetylcholine (ACh) and its precursor choline (Ch) in discrete brain areas of the rat. Rats were killed by microwave irradiation focused to the head at 0, 5, 10, 15, 20, 40 min and 1, 2, 3, 4 hrs after subcutaneous injection of an equitoxic dose (9/10 LD50) of soman (120 µg/kg) or DFP (2.25 mg/kg). The levels of ACh and Ch were assayed in brainstem (B), cerebral cortex (C), hippocampus (H), midbrain (M), cerebellum (R), and striatum (S) by gas chromatography/mass spectrometry (Anal. Biochem. 55: 438, 1973). Data were analyzed by a two-day between-subjects ANOVA (time x compound). ACh levels showed significant time-dependent elevation after administration of soman or DFP in all 6 brain areas. For ACh, significant elevations from control caused by soman were seen approximately 0.5 hr in B and R; 3 hrs in S; and more than 4 hrs in C, H, and M; while that caused by DFP was 0.5 hr in B and R; 1 hr in M; 2 hrs in C and H; and more than 4 hrs in S. Reliable differences between these two compounds were observed in areas C, M and S. Maximal ACh elevation was observed in C (↑ 320.3%) and H (↑ 94.3%) following soman and in C (↑ 110.4%) and R (↑ 130.6%) after DFP. A significant interaction of time x compound was found in areas C, M, R, and S. Ch levels also exhibited a significant time-course variation in C, M and R caused by either of these two compounds. Soman produced an increase of Ch for a duration of 2 hrs in C; 0.5 hr in M and 10 min in R while DFP's duration in these 3 areas was less than 30 min. The differences between soman and DFP were found to be significant in areas B, C, R, and S. Maximal Ch elevation was produced in C (↑ 257.5%) by soman and in M (↑ 110.2%) by DFP. A marked interaction of time x compound was observed in C, M, and R areas. These results indicate that the degree and duration of action of soman and DFP on cholinergic function differs in brain areas and that a generalized statement about the central effects of these two organophosphates could not be made.

221.3 EFFECT OF DEPOLARIZING AGENTS ON THE CA^{2+} INDEPENDENT, SPONTANEOUS RELEASE OF ACETYLCHOLINE FROM MOUSE FOREBRAIN MINCES. Paul T. Carroll, Department of Pharmacology and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

Minces of mouse forebrain were incubated for 5 min. in Krebs solutions containing varying concentrations of K^+ (4.7, 15, 35, 50 mM) in the absence or presence of extracellular Ca^{2+} and the amounts of choline and acetylcholine (ACh) released into the respective paraoxon containing media determined. Results indicated that the Ca^{2+} dependent but not the Ca^{2+} independent form of ACh release was increased as a function of K^+ conc. However, when minces were first incubated for 30 min in a normal Krebs solution containing the AChE inhibitor paraoxon and then incubated in Krebs solutions with varying concentrations of K^+, both Ca^{2+} dependent and Ca^{2+} independent forms of ACh release were augmented as a function of K^+ conc. This pretreatment did not alter the amount of Ca^{2+} dependent release of ACh elicited by any of the K^+ concentrations. Choline efflux was not enhanced by elevated K^+ from either the pre-incubated or control minces. Veratridine, like elevated K^+, stimulated only the Ca^{2+} dependent form of ACh release from minces not pretreated, whereas it stimulated both Ca^{2+} dependent and Ca^{2+} independent forms of ACh release from minces initially incubated in Krebs with paraoxon. Veratridine, unlike high K^+, stimulated choline efflux independently of Ca^{2+} from non-pretreated minces. Also, pretreatment of minces altered the ability of veratridine to enhance the Ca^{2+} dependent release of ACh from minces. These results suggest that under in vitro conditions, depolarization of brain tissue accelerates the release of Ca^{2+} dependent but not Ca^{2+} independent, spontaneous release of ACh. They also suggest that depolarization may enhance choline efflux from brain independently of Ca^{2+} if the pre-synaptic level of ACh has not been increased prior to depolarization. (Supported in part by NSF grant BNS-8117975).

221.4 MOLECULAR FORMS OF ACETYLCHOLINESTERASE IN THE DEVELOPING AND ADULT RAT BRAIN. Blair A. Ruff* and S. C. Sung (SPON: J. H. Quastel). Division of Neurological Sciences, University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Heterogeneity of the molecular forms of acetylcholinesterase (AChE) derived from brain tissue is well established. These molecular forms of AChE can be characterized by their sedimentation coefficients in sucrose gradients. In the present study, molecular forms of AChE were analyzed in the isotonic sucrose soluble fraction and the solubilized membrane fraction from brains of developing and adult rats. Density gradient centrifugation of isotonic sucrose soluble, or solubilized membrane-bound, AChE on gradients demonstrated 2 major peaks of enzyme activity with sedimentation coefficients of 10 S and 4 S. The membrane-bound AChE from adult rat brain was mostly composed of the 10 S form of enzyme with small activity of the 4S form; the 4S form being 7.1% to 20% of the 10 S form depending on the region of the brain. However, a much higher proportion of the 4 S form was found for the isotonic sucrose soluble fraction when compared with the membrane enzyme. In contrast to the membrane-bound AChE from adult rat brain, that from 6-day old rat contained a much higher proportion of the 4 S form of the enzyme. The sucrose soluble fraction from cerebellum of adult rat contained almost equal amounts of the 10 S and 4 S forms of AChE; however, that from striatum contained much more 10 S form than 4 S form.

The percentage of AChE activity, extracted by isotonic sucrose from the adult rat brain, was 11.3% from striatum and 30.5% from cerebellum. However, the percentage of AChE extracted by isotonic sucrose from 6-day old rat brain did not differ very much from region to region of the brain, e.g. 32.6% from striatum and 38.1% from cerebellum. The specific activity of AChE, on a protein basis, from membrane fraction of adult rat striatum was about 3 times that found in the sucrose soluble fraction. However, the specific activity in the cerebellum was nearly the same in both fractions. A similar pattern was observed in 6-day old rat brain. The specific activity in the cerebellum changed very little during development, while that observed in 6-day old rat striatum was about 23% of that of adult striatum.

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221.5 COATED VESICLES IN EMBRYONIC CHICK SKELETAL MUSCLE CONTAIN NEWLY SYNTHESIZED SECRETORY ACETYLCHOLINESTERASE. R.J.J. Benson* and R.E. Fine*(SPON: T.E. Benson). Depts. of Anat. and Biochem., Boston Univ. Sch. of Med., Boston, MA 02118.

Embryonic chick myotubes in culture synthesize and secrete acetylcholinesterase (AChE) into the culture medium (Wilson, B.W. et al., Dev. Biol. 33, 285-299, 1973). In order to establish if coated vesicles (CV's) play a role in the secretion of this enzyme, it was first necessary to ascertain if CV's isolated from embryonic chick skeletal muscle contain AChE. Sucrose-gradient-purified CV's were assayed for AChE activity by the colorimetric assay of Ellman et al. (Biochem. Pharmacol. 7, 88-95, 1961). Activity was found in the absence of inhibitors and in the presence of an inhibitor of pseudocholinesterase, iso-OMPA. This activity was abolished by an inhibitor of acetylcholinesterase, BW284C51. 0.1% Triton X-100 substantially increased activity as did freeze-thawing the CV's prior to assaying. These results suggest that the enzyme is contained within the vesicle. Electron microscopic AChE histochemistry (Karnovsky, M.J. and L. Roots, J. Histochem. Cytochem. 12, 219-221, 1964) of isolated CV's support this conclusion. When frozen-thawed CV's were pelleted, most of the AChE activity stayed in the supernatant. This indicates that the AChE in CV's is in a secretory and not an intrinsic membrane form. CV's were purified to homogeneity on a 0.15% agarose gel (Rubinstein, J.L.R. et al., J. Cell Biol. 89, 357-361, 1981) and the gel was stained for AChE activity by the method of Maynard, E. A. (J. Exp. Zool. 161, 319-335, 1966). The staining observed in the absence of inhibitors was abolished by BW284C51.

When cultured chick myotubes were treated with DFP, an irreversible inhibitor of cholinesterases, and allowed to recover in fresh medium for 2½ hours, AChE was found associated with CV's isolated from these cultures. Freezing, thawing and pelleting of these vesicles as above indicated that the enzyme was in a secretory form. 0.15% agarose gels of these CV's indicated that the activity was associated with the homogeneous band of CV's. Myotubes were also fixed and stained for AChE by the method of Karnovsky, M.J. and Roots, L., J. Histochem. Cytochem. 12,219-221, 1964). In thin sections of these cultures, stained CV's were found in cells treated with DFP and allowed to recover for 2½ hrs. and in cells not treated with DFP. No stained CV's were observed in cells fixed immediately after DFP treatment. Since it takes at least 3 hours for DFP-treated myotubes to recover their capacity to secrete AChE and for the enzyme to reappear at the cell surface (Wilson, B.W. and Walker, C.R., PNAS (USA) 71, 3194-3198, 1974; Rotundo, R.L. and Fambrough, D.M., Cell 22, 583-594, 1980), the activity associated with these CV's must represent newly synthesized enzyme.

221.6 CHOLINERGIC AGENTS MIMIC SEIZURE-RELATED ASPECTS OF KAINATE NEUROTOXICITY. J.W. Olney, T. deGubareff* and J. Labruyere*. Dept. of Psychiatry, Washington University Sch. Med., St. Louis MO 63110.

A distinctive pattern of acute brain damage involving limbic and related brain regions develops in adult rats as a consequence of sustained limbic seizures induced by systemic administration of kainic acid (KA) or dipiperidinoethane (DPE) or by intraamygdaloid injection of KA or folic acid (FA). This seizure-brain damage (S-BD) syndrome is of particular interest as it resembles the type of seizures and brain damage seen in human temporal lobe epilepsy. Certain behavioral effects of systemic KA or DPE resemble those of known cholinomimetics and the distribution of brain lesions induced by these agents follows a pattern potentially implicating central cholinergic pathways. Moreover, although the S-BD syndrome is not reproduced by intraamygdaloid injection of DPE, it is by an oxidized derivative (DPE-di-N-oxide) which structurally resembles the acetylcholine (ACh) agonist, oxotremorine (Olney et al., in press). Prompted by these observations and awareness that Wasterlain et al. have successfully used the ACh agonist carbachol for amygdaloid kindling, we injected known ACh agonists and cholinesterase (ChE) inhibitors into the rat amygdala and found that either class of agent reproduces the KA/FA type of S-BD syndrome. Of the agents tested, oxotremorine was the least and neostigmine the most consistently effective. The potency of the ChE inhibitor neostigmine in inducing seizure-related neuropathology is comparable to that of KA in that a 3 nmol intraamygdaloid dose of either agent is sufficient to trigger an S-BD syndrome. It should be noted that although intraamygdaloid FA, DPE-di-N-oxide or cholinergic agents all mimic the seizure-related aspects of KA neurotoxicity, none of these agents reproduces the direct neuron-necrotizing action that KA exerts locally when injected into brain.

Our findings signify that excessive activation of limbic ACh receptors (either by exogenous ACh agonists or synaptic accumulation of endogenous ACh) can lead to an acute seizure-linked neurodegenerative syndrome. Whether ACh mechanisms contribute to the seizure-related neurotoxicity of KA or FA warrants consideration as does the possibility that ACh mechanisms play a more important role in human epilepsy and epilepsy-related brain damage than has generally been appreciated. Intra-limbic application of cholinomimetics, especially neostigmine, may provide a useful animal model for studying mechanisms of and therapeutic approaches to epilepsy. For evidence that epilepsy and related brain damage can also be studied by systemic administration of cholinergic agents (provided rats are pretreated with lithium), see Honchar et al., Neurosci. Abst., 1982. Supported by grants from the Epilepsy Foundation of America and USPHS (NS-09156, DA-00259, RSA MH-38894 to JWO).

221.7 MONOETHYLCHOLINE MUSTARD AZIRIDINIUM ION, A CHOLINERGIC NEUROCHEMICAL PROBE: COMPARISON WITH CHOLINE MUSTARD AZIRIDINIUM ION. R.J.Rylett and E.H.Colhoun*. Dept. of Pharmacology, University of Western Ontario, London, Ontario, Canada N6A 5C1.

Monoethylcholine mustard aziridinium ion (MEChM Az), the N-ethyl analogue of choline mustard aziridinium ion (ChM Az), is reported to be an in vivo cholinergic neurotoxin (Fisher and Hanin, Life Sci.,27:1615,1980) and a potent inhibitor of synaptosomal high-affinity choline transport (Rylett and Colhoun,J.Neurochem., 34:713,1980). In our laboratory, ChM Az is employed as a neurochemical probe to study cholinergic function but the ubiquitous distribution of choline makes it difficult for it to be used as a control for comparison when analyzing effects attributable only to the presence and reactivity of the aziridinium ring (Az). We felt that MEChM Az, prepared as the nitrogen mustard counterpart of the choline analogue monoethylcholine, could serve better for comparison. In this report information about the chemistry, toxicity and in vitro actions of MEChM Az are detailed. MEChM Az, generated as the acetylated intermediate, was more stable in alkaline medium and at physiological pH than ChM Az suggesting that ethyl substitution on the nitrogen had a protective effect on the Az, at least from attack by hydroxyl ions. Toxicity studies showed the i.v. LD_{50} for MEChM Az to be 4.6 mg/kg and that for ChM Az to be 2.6 mg/kg. The i.p. LD_{50}'s for MEChM Az and ChM Az were 21.9 and 9.0 mg/kg, respectively. The mean of time to death for MEChM Az was longer than that observed for ChM Az, but there were no evident differences in the signs of toxicity with death attributed primarily to presynaptically-mediated neuromuscular blockade and respiratory failure. MEChM Az, like ChM Az, produced an irreversible, presynaptically-mediated blockade of neuromuscular transmission in the indirectly stimulated rat phrenic nerve-diaphragm preparation but the compounds were not equipotent, ChM Az being about twice as potent as MEChM Az. MEChM Az produced a time-dependent, irreversible inhibition of synaptosomal high-affinity choline transport. Evaluation of the kinetics of inhibition of uptake of choline by MEChM Az revealed a shift from initially competitive to noncompetitive blockade, as well as changes in the I_{50} concentration (decreasing with time). These parameters were qualitatively similar to ChM Az, with MEChM Az being one half as potent as ChM Az. The N-ethyl substitution on ChM Az to yield MEChM Az did not seem to produce qualitative differences in the actions of the drugs however small quantitative differences existed. Although parameters such as affinity for a site (ie. choline carrier) did not seem to be altered by the ethyl group, the reactivity of the Az may be changed with the monoethyl drug not forming complex bonds with nucleophilic groups as readily. Therefore both compounds may give the same endpoint but MEChM Az would take longer to produce it. (Supported by NRC Canada).

221.8 SYSTEMIC PILOCARPINE OR PHYSOSTIGMINE INDUCES SEIZURES AND BRAIN DAMAGE IN LITHIUM-TREATED RATS. M.P. Honchar, J.W. Olney and W.R. Sherman*. Dept. of Psychiatry, Washington Univ. Sch. Med., St. Louis, MO 63110.

Subcutaneous administration of either pilocarpine (Pilo, 30 mg/kg) or physostigmine (0.4 mg/kg) to rats which had received LiCl (3 meq/kg, sc) 24 hr earlier results in limbic seizures similar to fully kindled amygdaloid seizures and damage to several brain regions (e.g., the piriform, hippocampal and neocortices, the septum, amygdala and several thalamic nuclei). This syndrome of seizures and brain damage resembles that caused by systemic administration of kainic acid or intraamygdaloid injection of cholinomimetic agents (Olney et al., Soc Neurosci Abst, 1982). Li-treated rats which received atropine (150 mg/kg sc, n=6) 30 min before Pilo (30 mg/kg) as well as rats treated with Li alone (3 meq/kg, n=10) or Pilo alone (30 and 50 mg/kg, n=6,4) had neither seizures nor histopathology.

The administration of LiCl by itself causes a large increase in cortical D-myo-inositol-1-phosphate (MIP) which is a product of phosphoinositide metabolism (Sherman et al., J Neurochem 36, 1947, 1981). The Li-induced increase in MIP is blocked by atropine, thus it may reflect a cholinergic receptor-mediated change in the metabolism of CNS phosphoinositide lipids. The combined administration of Pilo and Li produces a remarkable enhancement of the MIP increase resulting from either agent alone (Table). The MIP increase, like the seizures, is blocked by atropine administration 30 min prior to Pilo (Table). A single 10 meq/kg dose of LiCl causes an increase in MIP comparable to that of combined Li and Pilo (Table) without inducing seizures (Munsell & Sherman, unpublished); however, it is known that a 6 meq/kg dose of LiCl reduces the seizure threshold to electroshock (Davenport, Am J Physiol 163, 633, 1950). The role of altered inositol lipid metabolism in seizure sensitivity and brain damage is under investigation.

Based on the results thus far obtained, the simultaneous administration of cholinomimetic agents, advertently or inadvertantly, along with lithium, warrants caution as well as further study. Supported by grants from USPHS (MH-14677, NS-05159, RSA MH-38894 to JWO) and from the Epilepsy Foundation of America.

TABLE: MIP in midline cerebral cortex (mmol/kg dry wt ± SEM)

Control	0.22 ± 0.01
Li (3 meq/kg)	0.85 ± 0.15
Pilo (30 mg/kg)	0.53 ± 0.06
Li + Pilo (3 meq + 30 mg/kg)	8.95 ± 1.34
Li + Pilo + Atropine (3 meq + 30 mg + 150 mg/kg)	0.77 ± 0.18
Li (10 meq/kg)	8.85 ± 0.61

221.9 ACETYLCHOLINE MIMICS THE SITE-SPECIFIC ENHANCEMENT OF DESYNCHRONIZED SLEEP SIGNS INDUCED BY CARBACHOL. H.A. Baghdoyan; M.L. Rodrigo-Angulo; R.W. McCarley and J.A. Hobson. Lab. Neurophysiol., Harvard Medical School, Boston, MA 02115

Cholinergic microstimulation of the pontine reticular formation (RF) with carbachol and bethanechol produces a marked enhancement of desynchronized (D) sleep signs. This enhancement is localized to the pontine RF and there is site specificity within the pons. We now report that direct injection of acetylcholine (ACh) and neostigmine (N) into the pons induces a dramatic increase in D sleep signs. Pending histological confirmation of the injection sites, we conclude that the effects of ACh are also site-specific within the pons.

Four cats were implanted with bilateral guide tubes aimed at the pons and with electrodes for recording the EEG, EOG, EMG and PGO waves. Two cats were used for pilot experiments and 2 were used in a systematic protocol. All drugs were injected unilaterally with a 1 ul Hamilton syringe in a volume of 250 nl sterile saline. Dosages were: ACh, 5 ug; N, 20 ug; carbachol, 4 ug; atropine, 15 ug. ACh and N were administered in a mixture (ACh/N). Polygraphic recordings were obtained for 4 hrs post injection.

In all four cats ACh/N produced enhancement of D sleep signs that was qualitatively and quantitatively similar to that of carbachol. In a pilot study the D sleep of one cat was increased six-fold after carbachol injection. ACh/N at the same site induced a seven-fold elevation of D sleep.

Carbachol in a second cat induced a syndrome characterized by state independent PGO wave activity. ACh/N also elicited continuous PGO wave activity when injected into the same site. The effect of ACh/N could be blocked with atropine.

Encouraged by these results we conducted a systematic study in two cats. D sleep was elevated to 275% of control (saline injection) levels following injection of ACh/N into the pons (N=10 injections). The number of D sleep episodes was doubled and the duration of the longest D sleep period was increased two and a half times after ACh/N. Cats were aroused easily from ACh/N-induced D sleep by auditory stimuli. Waking also was increased to 143% and slow wave (S) sleep was decreased to 57% of control values.

These data demonstrate that ACh, a naturally occurring neurotransmitter, can initiate and sustain D sleep signs, thereby reinforcing the concept of cholinergic D sleep generation.

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221.10 A SIMPLE, INEXPENSIVE, AND RAPID RADIO-RECEPTOR ASSAY FOR THE ESTIMATION OF BRAIN ACETYLCHOLINE. H.I. Yamamura, F.J. Ehlert* and W.R. Roeske*. Univ. of Arizona, Tucson, AZ 85724.

A simple, inexpensive, and rapid method for the estimation of brain acetylcholine (ACh) is described. The principle of the method is based on the high potency (IC_{50} = 5 nM) with which ACh inhibits the binding of the specific muscarinic agonist ligand, [^{3}H]cis methyldioxolane ([^{3}H]CD). The radio-receptor technique for the estimation of ACh involves three discrete steps: 1. preparation of muscarinic receptors, 2. preparation of experimental samples, and 3. performing the binding assay. A synaptosomal-mitochondrial fraction of cerebral cortex of male Sprague-Dawley rats was used as a convenient source of muscarinic receptors. To facilitate enzymatic hydrolysis of endogenous ACh, the homogenate was incubated at 37°C for 20 min and treated with paraoxon (10 uM) to prevent enzymatic hydrolysis of ACh during the binding assay. The homogenate was then treated with N-ethyl maleimide (1 mM) to increase specific [^{3}H]CD binding. Following these treatments, the homogenate was centrifuged twice and the pellet was frozen until use. For the determination of brain ACh, rats were killed by focused microwave irradiation. The cerebral cortex and corpus striatum were dissected, weighed, and homogenized. The homogenates were centrifuged and the supernatants were decanted and saved for use in the radio-receptor assay. Specific [^{3}H]CD binding was measured by a centrifugation technique. Aliquots of the cortical membrane preparation were incubated with [^{3}H]CD in a final volume of 2 ml containing 50 mM HEPES/Tris, pH 7.4 at 0°C. In some tubes, aliquots of tissue extract containing ACh were added, or aliquots containing known amounts of ACh were added for determination of a standard curve. The assay tubes were incubated at 0°C for 90 min. Following incubation, the tubes were centrifuged and the pellets were washed superficially with two aliquots of ice cold saline, dried and counted. Acetylcholine readily inhibited specific [^{3}H]CD binding with an IC_{50} of approximately 5 nM. Since the assay was carried out in a final volume of 2 ml, the amount of ACh causing 50% inhibition of specific [^{3}H]CD binding is about 10 pmoles. This value can be easily reduced to 2.5 pmoles if the assay volume is decreased to 0.5 ml. Specific [^{3}H]CD binding was much more sensitive to inhibition by ACh as compared to choline by a factor of 10,000. Acetylcarnitine (10 uM) did not interfere in our assay. The mean values for the ACh content of the cortex and striatum were 19.1 and 54.5 nmoles/g wet tissue weight, respectively, which agrees with published reports. The high sensitivity of the assay (1 pmole of ACh) is comparable to that of several other currently used chemical techniques, and thus, the method should have practical application to many experimental paradigms.

221.11 MUSCARINIC RECEPTOR REGULATION OF Ca^{++} TRANSPORT IN RAT SYNAPTIC MEMBRANES. D.H. Ross, H.L. Cardenas* and M.N. Monis*, Div. Molecular Pharmac., Univ. Tex. Hlth. Sci. Ctr., San Antonio, TX 78284

Presynaptic muscarinic receptors are believed to play a major role in the regulation of acetylcholine (Ach) release. The molecular mechanisms for Ach release and the modulation by muscarinic receptors are not known. Cytosolic Ca^{++} is believed to play a major role in triggering the release process, possibly by stimulating phosphorylation of membrane proteins. This Ca^{++}-dependent phosphorylation is known to be Calmodulin-dependent. Michaelson et al (PNAS 76:6336, 1979) reported oxotremorine reduced Ca^{++}-dependent phosphorylation of a 100,000 dalton protein as well as reducing Ach release from Torpedo synaptosomes. Atropine antagonized this response. We have studied the effects of muscarinic receptorinteraction with the synaptosomal membrane Ca^{++} pump to further define muscarinic receptor regulation of cytosolic Ca^{++} levels.

Rat synaptic plasma membranes were obtained by sucrose density gradient centrifugation. Ca^{++}-stimulated ATP hydrolysis and ATP-dependent Ca^{++} uptake were measured by $^{32}P_i$ release and $^{45}Ca^{++}$ uptake, respectively. Cytosolic Ca^{++} levels were approximated by Ca^{++}-EGTA buffers in the range of 0.1 to 5.0 μM. Ca^{++}-stimulated ATP hydrolysis was reduced from 150 nmoles P_i released/mg/min to 95 nmoles/mg/mn by incubation with 1 μM muscarine. Oxotremorine gave quantitatively similar results. Atropine (50 nM) completely inhibited the muscarine response while producing no inhibition at this concentration. Higher concentrations of atropine (1 μM) caused stimulation of Ca^{++}-ATPase in eserinzed preparations from 150 nM/mg/mn to 185 nM/mg/mn. The enzyme coupled response to ATP-dependent Ca^{++} uptake was also studied under similar conditions. Muscarine (10 μM) reduced ATP-dependent Ca^{++} uptake from 4.6 nm/mg/mn to 2.5 nm/mg/mn. Atropine (500 nM) significantly antagonized this response, with higher concentrations (1 μM) stimulating uptake.

These studies suggest that muscarinic receptors may be coupled to the Ca^{++} transport process in synaptic membranes. Inhibition by receptor agonists may alter the synaptosomal membrane mechanism for buffering cytosolic Ca^{++}. This may contribute to altered release of Ach in the presence of muscarinic agonists.

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221.12 MUSCARINIC CHOLINERGIC BINDING IN THE RAT OLFACTORY BULB FOLLOWING ADRENALECTOMY. N.S. Nadi* and D.C. Jimerson (SPON: E.S. Gershon) National Institute of Mental Health, Bethesda, Maryland 20205 and Department of Psychiatry, Wayne State University, Detroit, Michigan 48207

The main olfactory bulb (MOB) has a large number of mono- and polysynaptic projections to the limbic system (Broadwell, R.C. Neurosc. Symp. 3, 131, 1977). Among these areas are the hippocampus and the hypothalamus. Projections from the latter structure into (MOB) have also been shown. The electrical stimulation of the olfactory bulbs in the adult rat cause a significant increase in plasma corticosterone (Lecuona, F.A., et al., J. Endocr. 54 353, 1972). Conversely, the removal of the MOB causes a significant decrease in plasma corticosterone levels (Loyber, I., Neuroendocr., 13, 93, 1973/74). In view of these findings we wanted to investigate whether the disruption of the hypothalamic-pituitary-adrenal (HPA) axis by adrenalectomy had an effect on the MOB.

In this study we measured the properties of muscarinic cholinergic binding (affinity K_d, and number of sites B_{max}) to MOB membranes using ^{3}[H] quinuclidinyl benzilate (QNB). Male Sprague-Dawley rats were sacrificed 7 - 9 days following adrenalectomy (ADX), sham ADX, or adrenal medullectomy (ADMX). An additional group received ADX plus dexamethosone (DEX) (100 ug/day). ADX produced a significant decrease in B_{max} in comparison to controls (500 ± 124 vs. 1140 ± 130 fmol/mg protein, $p < .001$). This effect was blocked in ADX animals receiving DEX (B_{max} = 1200 ± 266 fmol/mg protein). No changes in binding affinity were noted. ADMS had no effect on ^{3}HQNB binding.

These results indicate that (HPA) axis modulates cholinergic activity in MOB. This effect may reflect altered activity of the previously demonstrated cholinergic afferents to MOB, although a direct effect on muscarinic receptor cannot be ruled out. These results complement data that altered activity of MOB can affect HPA function.

221.13 EFFECTS OF DFP AND OTHER DRUGS ON CHOLINERGIC NERVE FUNCTION IN THE RAT IRIS. J.S. Richardson, T.G. Mattio*, H.L. Bernstein-Goral*, and E. Giacobini. Lab. of Neuropsychopharmacology, Dept of Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT 06268.

The analysis of acetylcholine (ACh) levels, metabolism and release, as well as the uptake of choline, were performed on segments of rat iris to investigate the mechanisms involved in the response of the iris to acute and chronic cholinesterase inhibition. At various times after the topical administration of 0.1% DFP in sesame oil to the corneal surface, the rats were decapitated and the irises were removed. Pupil diameter was measured and ACh levels and cholinesterase activity were determined in each iris following extraction of remaining free DFP with chloroform. No changes were detected 1 min. after the DFP, but by 5 min., pupil diameter was reduced by 50%, esterase activity was reduced by 90%, and ACh was increased by 60%. There were no futher changes 30 min. after the DFP, but by 60 min., ACh had returned to control levels even though esterase activity was still inhibited by 90% and pupil diameter was still reduced by over 50%.

Choline is taken up by the rat iris by a low affinity process (KM=106.6 µM) and by a high affinity active transport system (KM=1.16 µM) that is temperature sensitive, sodium dependent, and is blocked in a dose dependent manner by hemicholinium in 10 µM or greater concentrations. Choline uptake is also reduced by millimolar concentrations of scopolamine and ouabain.

Electrical stimulation by 20 mA, 5 msec, 100Hz nearly square waves of isolated rat iris prelabled by incubation at 37C in Elliot's B buffer with tritiated choline, evokes a 1- to 2-fold increase in the release of tritium over the spontaneous release during pre-stimulation baseline. Scopolamine and DFP alter the release profile with 10 nM scopolamine increasing evoked release, 1 µM scopolamine increasing spontaneous release, and 1 µM DFP reducing both spontaneous and evoked release. These results are consistent with the existence of presynaptic muscarinic receptors that control the release of ACh from cholinergic nerve terminals in the rat iris.

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221.14 SUBCELLULAR DISTRIBUTION OF CHOLINE KINASE ACTIVITY IN RAT STRIATUM: EVIDENCE FOR MEMBRANE ASSOCIATED ENZYME. R.R. Reinhardt* and L. Wecker. Dept. Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

Evidence indicates that the enzyme choline kinase (EC 2.7.1.32), which catalyzes the conversion of choline to phosphorylcholine, is localized to the cytosolic fraction of rat brain, with little support for the presence of membrane associated enzyme activity. Our interest in the relationships among choline availability, transport and utilization for the synthesis of both phospholipids and acetylcholine led us to reinvestigate the subcellular distribution of this enzyme in rat striata. Enzyme activity in the cytosol from the crude mitochondrial fraction was 3.70 umoles/g initial wet weight/hr, as compared to the activity in the cytosol from the purified synaptosomal fraction which was 2.13 umoles/g initial wet weight/hr. When the crude mitochondrial preparation was fractionated, 67% of the total enzyme activity was found in the cytosol, while the osmotically shocked membranes were devoid of activity. However, when membranes were subjected to dissolution in Triton X-100, significant enzyme activity was noted, viz., 1.58 umoles/g initial wet weight/hr (29% of the total activity in the crude mitochondrial fraction). Since Triton X totally disrupts membranes, a less severe kaotrophic agent was used to determine how tightly the enzyme was bound to the membrane. Incubation of the membrane fraction with 1.5 M NaCl revealed an activity of 1.44 umoles/g initial wet weight/hr, similar to that obtained by total membrane solubilization. When the salt treated membrane was recentrifuged, 58% of the membrane associated activity was released into the supernatant while the remaining 38% was still present in the pellet. The amount of choline kinase releasable was a function of ionic strength and was linear between 0.1 and 1.5 M NaCl. Kinetic analyses indicated that the Km's for choline in the cytosolic, Triton-treated and salt released fractions were 0.74, 0.68 and 0.53 mM, respectively. Results indicate that choline kinase activity is associated with both the cytosolic and membrane fractions from rat striata. The ability to measure kinase activity in the presence of a high ionic strength gives evidence to suggest that the enzyme is bound to the membrane through an ionic interaction and not by a covalent bond or strong hydrophobic interactions. Although the specific function of the membrane associated enzyme has not, as yet, been determined, we suggest that it may play a regulatory role in determining the fate of choline as choline enters nerve terminals for use in the synthesis of phosphorylcholine and acetylcholine. (Supported by USPHS grants NIEHS-02278 and NIMH-33443.)

221.15 STIMULATION OF LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT VISUAL SYSTEM BY PHYSOSTIGMINE. E. D. London and M. Dam* Laboratory of Neurosciences, National Institute on Aging, Gerontology Research Center, Baltimore City Hospitals, Baltimore, MD 21224.

Two previous studies have used the functional mapping technique of Sokoloff et al. (J. Neurochem. 28: 897, 1977) to examine the effects of physostigmine (PHY), a reversible carbamate acetylcholinesterase inhibitor, on the regional uptake of [C-14]2-deoxy-D-glucose ([C-14]DG) by the rat brain (Nelson, S.R., et al., Brain Res. 157: 186, 1978; Friedland, R.P. and Meibach, R.C. Soc. Neurosci. Abs. 7: 494, 1981). In both of these studies, neostigmine produced no effects on [C-14]DG uptake, but PHY increased uptake in the superficial layer of the superior colliculus. In order to extend these findings, we examined PHY effects on local cerebral glucose utilization (LCGU) throughout the visual system of the rat brain, and compared PHY effects on LCGU with effects of the muscarinic agonist, oxotremorine (OXO).

Three month old male Fischer-344 rats were used for this study. Each rat was given one of the following treatments: control (saline, 1 ml/kg, i.p.), PHY (0.1 or 1 mg/kg of PHY salicylate, i.p., 20 min after atropine methylbromide, 1 mg/kg, s.c.), PHY (1 mg/kg, 20 min after scopolamine HBr, 1 mg/kg, i.p.). Saline and PHY were injected 20 min before [C-14]DG. OXO was injected 2 min before [C-14]DG. LCGU was determined as described by Sokoloff et al. (J. Neurochem. 28: 897, 1977).

Although 0.1 mg/kg of PHY produced no significant effects, 1 mg/kg of PHY dramatically stimulated LCGU in the superficial layer of the superior colliculus (122%), the nucleus of the optic tract of the pretectal area (126%), and the following components of the accessory visual system: the superior (49%), lateral (59%), and medial terminal (123%) nuclei, and the inferior fasciculus (100%). PHY did not significantly affect LCGU in the visual cortex or in the dorsal nucleus of the lateral geniculate body. Scopolamine did not significantly antagonize the stimulatory effects of PHY in components of the visual system.

OXO increased LCGU in the superficial layer of the superior colliculus (93%) and in the nucleus of the optic tract (57%) and produced nonsignificant increases in some components of the accessory visual system. All OXO effects on LCGU were blocked by prior treatment with scopolamine. These results indicate that cholinergic drugs can influence function in the rat visual system. The fact the PHY effects on LCGU are not antagonized by scopolamine indicates that they result from nicotinic rather than muscarinic actions of the drug. A nicotinic rather than muscarinic action in the accessory visual system is further supported by the relative lack of effect of OXO in these brain regions.

221.16 CHOLINERGIC DEPOLARIZATIONS OF CENTRAL NORADRENERGIC NEURONS. T. M. Egan,* J. T. Williams* and R. A. North (SPON: T. Weiss). Neuropharmacology Laboratory, Department of Nutrition & Food Science, M.I.T., Cambridge, MA 02139.

There is a high concentration of cholinergic muscarinic binding sites on locus coeruleus (LC) neurons, and iontophoretic application of ACh increases the firing rate of rat LC neurons recorded with extracellular electrodes in vivo. Intracellular recordings were made from neurons of rat LC in a 300 µm slice of pons. Drugs were applied either by superfusion or by pressure from a fine tipped pipette above the slice surface. Superfusion with acetylcholine (ACh) depolarized LC neurons; this was associated with an increase in input resistance. Pressure ejection of ACh (pipette concentration 100 µM - 10 mM; 10 - 100 ms at 5 - 20 psi) caused a biphasic depolarization comprising an early component of rapid onset and decay (duration 1 - 2 s) and a slower, longer lasting component (duration 10 - 30 s). These depolarizations, were both potentiated by physostigmine (100 nM), and persisted in Ca^{++}-free high-Mg^{++}solutions. The fast component was blocked by hexamethonium (400 µM), the slow component was blocked by hyoscine. The antagonism by hyoscine of the slow ACh depolarization was competitive with a pA_2 of 8.5 $\pm$ 0.1 (S.D., n= 5). The results indicate that ACh has both nicotinic and muscarinic actions on brainstem noradrenergic neurons.

221.17 METRIZAMIDE EFFECTS ON CHOLINERGIC MECHANISMS. M. R. O'Neil*, E. Marder, and R.I. Grossman *(SPON: J.C. HALL). Biology Department, Brandeis University, Waltham, MA 02254 and Radiology, Hospital of University of Pennsylvania, Philadelphia, PA 19106.

Metrizamide is a water-soluble iodinated compound (m.w. 789). It is used clinically for many neuroradiological procedures such as myelography and cisternography during which it may reach local concentrations as high as 100-130mM. It is also commonly used as a medium for density gradient separations of cells and macromolecules at concentrations of 200-400mM. We have found that metrizamide produces concentration-dependent: 1) inhibition of purified eel acetylcholinesterase (AChE) (E.C.3.1.1.7) activity 2) reduction in the amplitude of postsynaptic acetylcholine (ACh) responses.

AChE activity was assayed colorimetrically by the method of Ellman et al. (Biochem Pharmacol, 7: 88-95, 1961), in the presence of metrizamide concentrations from 10mM to 150mM. 50% inhibition was produced by about 85mM metrizamide. 50mM metrizamide decreased enzyme activity 35-40% at substrate concentrations between 0.06mM and 0.94mM, indicating the inhibition is not competitive.

The cholinergic gm1 muscles of the crab, Cancer irroratus (Marder & Paupardin-Tritsch, J exp Biol 88: 147-159, 1980) were used for electrophysiological experiments. Muscle fibers were impaled with two 5-10 MΩ KCl-filled microelectrodes and ACh iontophoretically applied to the surface of the fiber. Reduction in the amplitude of the iontophoretic ACh response recorded under both current-clamp or voltage-clamp conditions was seen at metrizamide concentrations as low as 10mM (26% reduction in ACh response amplitude). 25mM produced ~50% decrease, and 50mM ~66% decrease in response amplitude. No change in muscle fiber input impedance was measured at these metrizamide concentrations. Equivalent decreases in amplitude of neurally-evoked cholinergic Excitatory Junctional Potentials (EJPs) were also seen. However, companion experiments on the glutamergic gm6 muscle showed no effect of 50mM metrizamide on glutamate-mediated EJPs.

These data may help explain some of the side-effects associated with the clinical use of metrizamide, and show that high concentrations of metrizamide are not inert on biological tissues.

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221.18 CHOLINERGIC STIMULATION OF HIPPOCAMPAL PYRAMIDAL CELLS IS INHIBITED BY INCREASING MEMBRANE CHOLESTEROL.. Fulton T. Crews, Arturo Camacho* and M. Ian Phillips. Departments of Pharmacology and Physiology, University of Florida Medical School, Gainesville, FL 32610

Cholinergic agonists act on muscarinic receptors to directly excite CA1 pyramidal cells in the hippocampus. Since increases in the cholesterol content of synaptosomal membranes occur during chronic ethanol consumption and during aging, we investigated the effects of cholesterol incorporation on carbachol stimulation of CA1 pyramidal cells in slices of rat hippocampus. Brain slices were prepared from 200-300 g albino rats and maintained by constant perfusion with a high O_2 solution at 37°C for several hours. Recordings of frequency of firing were made by glass micropipettes (3-10 Mohm) and carbachol (1 mg/ml) applied by pressure injection. Cholesterol was incorporated into slices by perfusion with a buffer containing bovine serum albumin (BSA) previously saturated with cholesterol by sonication. In each experiment slices were perfused with fresh buffer for a two hour equilibration period. After equilibration several control responses to carbachol were obtained from a pyramidal cell near the surface of the slice. When the control response was established the perfusion buffer was changed to an identical buffer containing BSA saturated with cholesterol. This change in buffer had no apparent effect on basal firing and no immediate effect on the carbachol response. However, after perfusing with cholesterol containing buffer for a few minutes the response to carbachol was reduced and after approximately 30 minutes there was almost complete inhibition of the response. Once maximal inhibition was achieved the buffer was changed to a buffer containing lipid free BSA with no cholesterol. Continuous perfusion with this wash-out buffer for about 90 minutes restored carbachol responsiveness to control levels. To determine the extent of cholesterol incorporation into slices, experiments were done using [^{3}H]-cholesterol. [^{3}H]-cholesterol was rapidly incorporated into the slice during perfusion with cholesterol containing buffer and was slowly removed by the wash-out buffer. The time course for the alteration in the carbachol response corresponded with the incorporation and removal of [^{3}H]-cholesterol. It is possible that changes in neuronal membrane composition and fluidity interfere with cholinergic nerve transmission and thereby contribute to the memory deficit suffered with increasing age and following chronic alcohol intoxication.

221.19 PEPTIDERGIC AND CHOLINERGIC ACTION ON THE HIPPOCAMPAL BRAIN SLICE. Arturo Camacho* and M. Ian Phillips. Department of Physiology, University of Florida Medical School, Gainesville, FL 32610.

The hippocampus has been proposed as an important brain site involved in memory function based on lesioning and neurological studies. Peptides and cholinergic transmission have also been implicated in memory based on their effects in behavioral studies. In this study, the acetylcholine agonist, carbachol, and the peptides adrenocorticotrophic hormone (ACTH fragments), vasopressin (AVP), and AVP analog desaminocys, D-Arg vasopressin (DDAVP), thyrotropin releasing hormone (TRH), cholecystokinin (CCK8) and oxytocin were tested for their direct and synaptic activity on pyramidal cells of the CA1 region in hippocampal brain slices in vitro. Doses were 10^{-9} M to 10^{-6} M.

In 292 units in the CA1 region carbachol and AVP predominantly excited neurons (see Table). Firing rates increased from 5-10 per sec to 60 per sec in bursts of activity. The onset was 10-30 sec to the new firing rate. All responses showed recovery and were reversible. ACTH excited 33% of the

Region Peptidergic and Cholinergic Effects on Hippocampal CA1

Agent	n	% Excitation	% Inhibition	% No Effect
AVP	62	81	0	19
Carbachol	104	95	0	5
DDAVP	23	48	0	52
Oxytocin	29	52	31	17
ACTH	60	33	0	67
TRH	10	0	100	0

cells. There was no difference between $ACTH_{1-0}$, $ACTH_{4-10}$ and $ACTH_{4-7}$. Oxytocin and CCK8 showed both excitatory and inhibitory effects. Atropine blocked carbachol, but did not block the peptide effects; TRH inhibited cells only. The vasopressin antagonist blocked the effects of AVP, but not the effects of either carbachol or oxytocin. Oxytocin and AVP both excited some neurons and acted independently on others. Lowering calcium (1 mM)/magnesium (1.5 mM to 15 mM) ratios or adding manganese (2 mM) to the perfusing solution did not block these effects. This implied that the effects were direct and not synaptically mediated. The excitatory action of acetylcholine, AVP and oxytocin correlate with the proposed behavioral role of these agents in memory function.

221.20 VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS INFECTION (VEE) AND ACETYLCHOLINE METABOLISM. E. Bonilla, H. Hernandez*, M. Salazar*, and P. Rangel*. Instituto de Investigaciones Clínicas, Facultad de Medicina, Universidad del Zulia, Zulia, Venezuela.

Sprague Dawley male rats and Swiss male albino mice were innoculated intraperitoneally with a suspension containing respectively 1,000 and 100 lethal doses 50 of Guajira strain of VEE virus. The control and disease animals were killed by decapitation 6-8 days after innoculation when the latter presented evidence of encephalitis. Each brain was quickly removed and the neostriatum, midbrain, hippocampus, hypothalamus, frontal cortex, pons and cerebellum were immediately dissected out at 4° centigrade and stored at -80° C until analyzed for choline acetyltransferase (CAT) and acetylcholinesterase (ACE) acivities. The CAT activity was significantly decreased in the caudate nucleus, hypothalamus, midbrain, hippocampus and frontal cortex of both mice and rats after VEE innoculation. The decrease in activity was only detected when clinical signs of the disease were evident. ACE activity was not affected in any of the 7 brain regions analyzed in mice and rats. In a few surviving experimental rats sacrificed 3 months after the viral infection, no alterations could be detected in the activities of CAT, tyrosine hydroxylase (TH) and glutamate decarboxylase (GAD) in any of the brain regions, in spite of the very high hemoglutination inhibition antibody titers found in the animals. These observations lend support to the assumption that inhibition of synthesis of new enzymes and/or enzyme inactivation are mechanisms whereby the alterations in CAT activity as well as the previously reported decreases in activity of TH (Neurochemical Research Vol. 6, page 691, 1981) and GAD (Neurochemical Research Vol. 5, page 209, 1980) could be produced during the acute phase of the viral infection.

222.1 OPIATE-RECEPTOR BLOCKADE REDUCES VOLUNTARY RUNNING BUT NOT SELF-STIMULATION IN HAMSTERS. C.D.Potter*, K.T.Borer and R.J. Katz*. (SPON: C.J.D'Amato) Department of Physical Education,Univ.of Michigan,Ann Arbor,MI 48109 and Department of Psychology, Johns Hopkins University,Baltimore,MD 21218.

Naltrexone, a long-acting opiate receptor blocker was administered to female hamsters at two doses, 10 and 20 mg/kg,I.P. prior to 12 hr of nocturnal running or every 12 hr during access to hypothalamic self-stimulation to determine whether the endogenous opiates played a role in either of these two motivated behaviors. In addition, we wanted to determine whether the neuroendocrine facilitation of somatic growth, which is induced by voluntary running in hamsters (Borer,K.T. and Kelch,R.P.,Amer.J.Physiol 234:E611,1978) also occurred in response to intracranial self-stimulation in this species.

Ten adult female hamsters were implanted with intracranial self-stimulation electrodes.Five of these animals engaged in stable self-stimulation behavior,while the other five served as controls. Hamsters were exposed to self-stimulation during 30 days.Each dose of naltrexone or saline was administered on four successive days in random order.Five of the hamsters used in self-stimulation experiment were subsequently exposed to two weeks of voluntary running on horizontal activity discs.Each dose of naltrexone or saline was administered on two occasions in random order. Naltrexone suppressed total running activity, running speed, and increased total pause time in a dose-dependent fashion during the first six hours post-injection, and these effects were present at the higher dose level during the 12 hours post-injection. Naltrexone did not affect the number of times the running bouts were initiated, nor the rate of hypothalamic self-stimulation. Furthermore, the weight gain was unaffected by four weeks of self-stimulation, but was significantly greater during the two weeks of voluntary running.

We conclude that the stimulation of endogenous opiate receptors in mature female hamsters: (1) helps support high levels of voluntary running, possibly by enhancing the incentive properties of this motivated behavior; (2) plays no apparent role in the initiation of running bouts, or (3) in maintenance of intracranial self-stimulation in the lateral hypothalamic area;and (4) may contribute to facilitation of somatic growth that accompanies spontaneous running but not the hypothalamic self-stimulation in this species.

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222.2 ELECTROPHYSIOLOGICAL EVIDENCE FOR MORPHINE EXCITATION OF VENTRAL TEGMENTAL AREA DOPAMINE NEURONS. R.T. Matthews and D.C. German. Depts. of Physiol. & Psychiat., Univ. of Texas Health Science Center, Dallas, Texas 75235.

Immunocytochemical and receptor binding experiments have indicated opiate-like substances and opiate receptors in close association with substantia nigra (A9) and ventral tegmental area (A10) dopamine (DA) neurons. Pharmacological evidence indicates that DA turnover in nigrostriatal and mesolimbic/mesocortical DA terminals increases after systemic morphine. Furthermore, A9 DA neuronal impulse flow increases after systemic morphine. However, iontophoresis of morphine onto A9 DA neurons has little effect (2-7% decrease in firing rate) (Pert et al, Catecholamines: Basic & Clinical Frontiers, p. 1041, 1979). The present experiment sought to compare the effects of systemic and iontophoretically applied morphine on DA unit activity in the A10 and A9.

Male albino rats (200-300 g) were anesthetized with chloral hydrate or were paralyzed, and unanesthetized. Standard single unit recording methods were used with either metal or 5-barrel micropipettes. The firing rates of both A9 (3 of 3 cells) and A10 cells (9 of 12 cells) were increased by i.v. morphine (0.1 - 10.0 mg/kg), but A10 cells were 2-5 times more sensitive than A9 cells. A10 firing rates were increased more than A9 (up to 100% vs. 30%). These increases were reversed by naloxone (0.1 - 0.5 mg/kg, i.v.). Of the 8 cells in A9 tested with iontophoresed morphine (0.1 M, pH 4.0, 10-30 nA), 3 cells had their firing rates slightly decreased (18-25%), one was increased (35%) and 4 were unaffected. Twenty-four A10 cells were examined; 13 were excited (up to 80%), 2 were inhibited (12-49%), and 9 were unaffected. In the pars lateralis 5 of 5 cells were excited (20-100%). The effect of iontophoresed morphine often outlasted the period of drug application as previously reported in the locus coeruleus (Bird & Kuhar, Brain Res., 122: 523, 1977). Both systemic and iontophoresed morphine frequently increased the activity of A10 cells to the point of apparent depolarization block. Thus, if a hyperpolarizing substance, like DA or GABA, was simultaneously iontophoresed, the cell began firing again. This effect was most often observed in the anesthetized preparation. Systemic naloxone (1-2 mg/kg) blocked the effects of iontophoresed morphine.

These data suggest that a population of A10 and pars lateralis DA neurons have excitatory opiate receptors. The reinforcing and locomotor effects produced by direct infusion of morphine into the A10 region may be due to the excitation of the A10 DA neuron.

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222.3 DIFFERENTIAL CARDIOVASCULAR AND RESPIRATORY EFFECTS OF OPIOID PEPTIDES MICROINJECTED INTO HINDBRAIN NUCLEI OF THE RAT. A.H. Hassen, G.Z. Feuerstein, A.I. Faden, Neurobiology Research Unit, Uniformed Services University, Bethesda, MD 20814

Intracisternal administration of opiates has been shown to elicit hypotensive as well as hypertensive responses. Tachycardia and bradycardia have also been observed. These responses may be due to the presence of multiple opiate receptors within different brain stem nuclei which mediate differential cardiovascular and respiratory functions. In order to clarify the role of μ- and δ-opiate receptors in cardiorespiratory regulation, relatively specific μ- and δ- agonists were microinjected (0.1 μl) into the Nucleus of Tractus Solitarius (NTS) and the Lateral Reticular Nucleus (LRN) of pentobarbital anesthetized rats. The μ-agonist, D-Ala^2-MePhe^4, Gly-ol^5 enkephalin (DAGO), and the δ-agonist, D-Ala^2, D-Leu^5 enkephalin (DADL), were administered via a glass capillary (30 μ) implanted stereotaxically into the caudal NTS at the level of the obex (L:0.5 mm; V:-0.5 mm) and the LRN 0.3 mm rostral to the obex (L:1.9 mm; V:-2.3 mm). The injection sites were identified microscopically and diffusion of drug estimated by fast green and ^{3}H-DADL. Blood pressure, heart rate and respiratory rate were monitored for 60 min after saline or 3-300 pMol of DAGO or DADL. In the NTS, DAGO caused a dose-dependent increase in systolic BP:+11±5 mmHg, +24±3 mmHg, +37±8 mmHg (P <.01); diastolic BP was increased only by the highest dose. The pressor response was accompanied by a 36±4 to 63±3 Beat/Min increase in heart rate (P <.01). Respiratory rate was increased by the lower doses (+12±3 and +16±2 Breaths/Min) and depressed (-10±6 Breaths/Min) by the high dose of DAGO. DADL also elicited dose-dependent pressor responses but the EC_{50}DAGO/DADL=28/1. The δ-agonist caused tachycardia (up to 53±5 Beats/Min, P <.01) and stimulation of respiratory rate after each dose. Naloxone (0.1 mg/kg, i.v.) reversed all of the responses elicited by the peptides but had no effect on saline treated rats. In the LRN, DAGO (30 and 300 pMol) depressed mean BP by -24 and -37 mmHg, respectively, and respiratory rate by -26 and -35 Breaths/Min, respectively. DADL elicited similar responses but was less potent than DAGO. The results suggest that μ receptors mediate opposite cardiovascular and respiratory effects in two different brain stem nuclei: excitatory in NTS and suppressor in LRN.

222.4 MORPHINE FACILITATES THE ACTIVITY OF DOPAMINERGIC NEURONS IN THE RAT VENTRAL TEGMENTAL AREA. K. Gysling* and R. Wang (SPON: T.C. Westfall). Dept. of Pharmacol., Sch. of Med., St. Louis Univ., St. Louis, MO 63104.

It has been reported that morphine increases dopamine (DA) turnover in the nigrostriatal DA system. Systemic administration of morphine increases the rate of spontaneous firing of DA neurons localized in the substantia nigra (A9) (Iwatsubo and Clouet, J. Pharmacol. Exp. Ther. 202, 429, 1977). It has been proposed that the stimulatory effect of morphine upon A9 DA cells largely depends on the integrity of the striatonigral pathways. Johnson et al. (Brain Res. 194, 566, 1980) have demonstrated the presence of enkephalin terminals and fibers in close relation to the DA neurons of A9 and ventral tegmental area (A10). Thus, it was of interest to study the effect of morphine in the spontaneous electrical activity of A10 DA neurons. For comparison, morphine effects on A9 DA cells were also studied.

Extracellular recording of A9 and A10 DA neurons were carried out in rats under chloral hydrate (400 mg/kg i.p.) anesthesia. Morphine (1-3 mg/kg i.v.) produced a marked increase in the spontaneous firing of both A9 and A10 DA neurons. Naloxone (0.1 mg/kg i.v.) reversed these effects. In order to examine whether the observed effect with morphine depends on possible afferents or feedback pathways to A9 and A10 DA neurons, the following lesion studies were performed: (1) radiofrequency lesion of the dorsal and medial raphe nuclei (7 days prior to experiments), and (2) transection of the medial forebrain bundle (both acute and chronic). Lesion of midbrain raphe nuclei did not interfere with the observed morphine effect on A9 and A10 DA neurons. The same is true for the morphine effect upon A10 DA cells after transection of the medial forebrain bundle. However, a large number of A9 DA neurons no longer responded to morphine after the transection of the medial forebrain bundle. To decide whether morphine has a direct action on DA neurons, morphine was iontophoretically applied on A9 and A10 DA neurons. On both A9 and A10 DA neurons, morphine significantly increased the spontaneous activity. However, these effects were not reversed by naloxone. The specificity of this morphine effect in terms of the opiate receptor(s) involved remains to be established. It is confirmed that morphine effect on A9 DA cells largely depends on the striatonigral feedback pathways. On the other hand, it appears that the facilitatory effect of morphine upon A10 DA neurons does not depend on afferents or feedback pathways from the forebrain. It is still possible that the morphine effect observed depends on afferents arriving from regions of the CNS posterior to the VTA. However, the results of the iontophoretic studies suggest that morphine may have a more direct action on cells in the ventral tegmental area. (Supported by USPHS Grants MH-34424 and MH-00378)

222.5 THE EFFECTS OF NEW, DELTA-SELECTIVE DIMERIC ENKEPHALINS ON REGULATION OF CYCLIC AMP LEVELS IN NG108-15 CELLS. S. A. Krumins* and D. Rodbard* (SPON: G. Guroff). Biophysical Endocrinology Section, ERRB, NICHD, Bethesda, MD 20205.

Dimeric Pentapeptide Enkephalin (DPE_2), consisting of two molecules of [D-Ala^2,Leu^5]enkephalin linked at the C-terminal with ethylenediamine (synthesized by Y. Shimohigashi, Mol. Pharm. 21: 558, 1982), has been characterized in both δ- and μ-specific binding assays using NG108-15 cells and rat brain. This dimer shows a preferred δ-receptor specificity, and exhibits six-fold higher affinity ($K = 47x10^8\ M^{-1}$) for the receptors on NG108-15 cells than the δ-specific monomer [D-Ala^2,D-Leu^5]enkephalin (DADLE, $K = 8x10^8\ M^{-1}$). Increased affinity of the bivalent DPE_2 appears to be due to bridging of two closely spaced δ-receptors. Binding affinity of DPE_2 is modulated to a greater extent by guanine nucleotides and cations than that of DADLE. The presence of 3 mM of $Mn^{2+}$and 50 mM of Na^+ resulted in 52% and 33% decrease in affinity of DPE_2, respectively. In contrast,the presence of 0.02 mM of GTP increased affinity up to 52%. Affinity of DADLE showed only small changes.

Based on mouse vas deferens assays (T. Costa) and studies measuring inhibition of adenylate cyclase (a.c.) activity in prostaglandin E_1 (PGE_1,10 μM)-stimulated NG108-15 cells incubated for 30 min with enkephalins, DPE_2 was assessed to be an agonist and equipotent with DADLE in the a.c.-assay (IC_{50} = 0.16 nM). In contrast to the 80% decrease in cAMP levels in PGE_1-stimulated cells and the apparent equipotency of DPE_2 and DADLE after 30 min incubation, prolonged incubations of 20-100 h with either DPE_2 or DADLE resulted in two-fold increased cAMP levels and an apparent differential effect on the time course of maximum cAMP-production: DPE_2 exhibiting a longer lasting increase than DADLE. This differential effect was not due to different rates of degradation. The effect of another new, δ-selective dimer, Dimeric Tetrapeptide Enkephalin [D-Ala^2,desLeu^5-NH(CH)$_6$]$_2$ ($DTE)_{12}$, and several enkephalin monomers on cAMP regulation was also studied.

CONCLUSION: The enkephalin dimers are potent agonists, which inhibit adenylate cyclase activity after short incubations. The dimers appear to differ in binding in the presence of cations and GTP and effect on enzyme activity after prolonged incubations from enkephalin monomers.

222.6 MORPHINE TOLERANCE INDUCES AN ENDOGENOUS OPIATE ANTAGONIST IN CEREBROSPINAL FLUID (CSF). G. Q. Lu*, J. N. Johannessen*, and D. J. Mayer. Department of Physiology, Medical College of Virginia, Richmond, VA 23298.

Despite intensive investigation, the nature of tolerance to opiates remains enigmatic. One possible mechanism is that introduction of exogenous opiates results in production by the nervous system of a compound(s) which antagonizes opiates. If the nervous system were to produce such an antagonist in response to chronic opiate administration, then such a compound might be released into the CSF.

In order to examine this possibility, CSF was withdrawn from rats chronically treated with either morphine or saline and immediately injected into the intrathecal space of untreated rats. This was followed by an analgesic dose of morphine delivered intrathecally. As shown in Figure 1, infusion of CSF from morphine tolerant rats resulted in a complete antagonism of morphine analgesia. CSF from saline treated animals was without effect on morphine analgesia. Rats pretreated with morphine tolerant CSF did show eventual development of morphine analgesia, presumably due to metabolism or clearance of the antagonistic factor. Morphine tolerant CSF is, by itself, without effect.

The identity of the compound(s) responsible for this potent effect is still under investigation, but it appears to be stable at room temperature and when frozen. Importantly, this factor is specific to morphine tolerance since it is absent in the CSF of barbiturate tolerant rats.

These experiments demonstrate that chronic morphine administration results in the presence in CSF of a compound which potently antagonizes the acute analgesic effects of morphine. Such a compound is likely to be found in considerably higher concentration within neural substrates mediating opiate effects. If true, this would provide a simple explanation of morphine tolerance.

Supported by PHS grant DA 00576 to DJM.

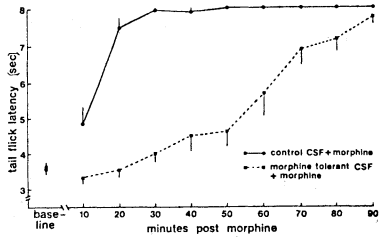

Figure 1

Effect of morphine tolerant CSF on morphine analgesia

222.7 BIOCHEMICAL SUPERSENSITIVITY OF OPIATE RECEPTORS AFTER CHRONIC BLOCKADE IS ASSOCIATED WITH FUNCTIONAL SUPERSENSITIVITY OF CARDIOVASCULAR BUT NOT RESPIRATORY RESPONSES TO MORPHINE IN SHR RATS. A.Pfeiffer*, D.G.Pfeiffer*, G.Feuerstein, A.Faden, and I.J.Kopin Lab.Clin.Sci.,NIMH and Dept.of Neurobiol., USUHS, Bethesda 20205.

Although an increase in the number of brain opiate receptors upon chronic blockade by naloxone is well established (Schulz et al., Arch.Pharmac.(1979) 306,93-96), functional supersensitivity of central opiate receptors remains to be demonstrated. In spontaneously hypertensive rats (SHR) a naloxone (nal) reversible analgesia suggests an enhanced endogenous opiate activity which might be associated with the development of hypertension (Zamir et al., Brain Res.(1980) 184, 299-310). We studied cardiovascular and respiratory responses to morphine 1 day after 4 weeks of treatment with nal (2mg/h s.c. via osmotic minipumps) of SHRs between 4-8 weeks of age, i.e. during the onset of hypertension.

Naloxone treated SHRs gained significantly less weight (199±5 vs 157±5g, $p<0.001$).Blood pressure (BP) and heart rates (HR) of treated and control rats were not significantly different at the onset (BP in mm Hg:111±6 controls, 111±3 nal; HR in beats/min:451±11 controls, 437±11 nal) or after termination of treatment (BP:144±4 controls, 141±4 nal; HR: 349±16 control, 406±20 nal).The awake rats were challenged with 1 and 10 mg/kg morphine (mor) i.a.and cardiovascular and respiratory parameters were monitored 20 min thereafter:

	HR change (beats/min)		BP change (mm Hg)	
morphine	controls	nal treated	controls	nal treated
1 mg/kg	-16±11	-68±17*	-2.4±2	-10.2±3
10 mg/kg	32±15	-26±16*	10.5±3	-13.7±6*

Naloxone treated rats were significantly (*=$P<0.05$) more sensitive to cardiovascular depressant effects of 1 or 10 mg/kg mor. However there was no difference in respiratory parameters; i.e. pH, pCO_2 and pO_2 were not significantly affected by 1mg/kg mor and a pronounced hypoxia, hypercapnia and acidosis occurred in both groups after 10 mg/kg of morphine. Opiate receptor binding sites were increased by 80% in the anterior hypothalamus and by 100% in the brainstem of nal treated vs control SHRs. Mu and delta sites were similarly increased in number without apparent change in affinity.

In conclusion, these data suggest that (a)endogenous opiates participate in the control of body weight in SHRs (b)opiates are not required for development of hypertension (c) opiate receptors involved in cardiovascular function differ from the ones involved in respiratory function (d) biochemical and cardiovascular opiate supersensitivity are associated (e)cardiovascular depressant effects of opiates are not dependent on respiratory depression as observed after 1 mg/kg of mor in nal treated SHRs.

222.8 EVIDENCE FOR ADRENAL MEDULLARY OPIOID INVOLVEMENT IN STRESS ANALGESIA. J.W. Lewis, M.G. Tordoff, J.C. Liebeskind, and O.H. Viveros, Dept. of Psychology, University of California, Los Angeles, CA 90024 and Dept. of Medicinal Biochemistry, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Prolonged footshock stress causes analgesia in the rat that is antagonized by the opiate antagonist naloxone, develops tolerance upon repeated exposure, and thus appears to be mediated by opioid peptides. Brief footshock stress also causes analgesia but this response is nonopioid in nature (Lewis et al., 1980; 1981). Findings that adrenal demedullation or denervation severely reduce only the opioid form of stress analgesia (Lewis et al., 1981), led us to suggest that enkephalin-like peptides of the adrenal medulla may be important mediators of this phenomenon. We now report biochemical evidence in support of this hypothesis.

Adrenal medullae were obtained from: A) Rats sacrificed immediately after exposure to either prolonged (2.5 mA, 1 sec on/5 sec, 20 min), brief (2.5 mA, on for 3 min), or no footshock stress; B) Rats subjected to chronic prolonged or brief footshock (daily for 14 days) and sacrificed either immediately or 24 hr after the last stress session; and C) Rats treated with reserpine (2 mg/kg) on two successive days and subjected to prolonged or no footshock stress 24 hr after the last injection and immediately prior to sacrifice. Opiate-like material in the adrenal medulla was quantified using a receptor binding assay (Chang & Cautrecasas, 1980), and catecholamine content was determined fluorometrically (Anton & Sayre, 1962).

Prolonged, but not brief, footshock stress caused a significant depletion of adrenal opioids ($p < .01$, compared with nonstressed controls). After 14 exposures, prolonged footshock no longer reduced adrenal opioid content, measured immediately or 24 hr after the last footshock session. This corresponds with our observation that tolerance to opioid stress analgesia occurs after 14 sessions (Lewis et al., 1981). Chronic brief stress did not affect adrenal opioids. Reserpine treatment, as reported in other species (Viveros et al., 1980), was found to reduce the catecholamine content but to increase dramatically the content of opiate-like material in the rat adrenal medulla ($p < .05$, compared with controls in each case). We have previously shown that prolonged footshock causes a potentiated analgesia in animals treated with this drug (Lewis et al., 1981). These biochemical data, taken together with our behavioral observations, provide compelling evidence for the involvement of adrenal enkephalin-like peptides in the mediation of opioid stress analgesia. (Supported by NIH grant NS07628 and Wellcome Research Laboratories.)

222.9 *In vivo* electrochemical evidence for a presynaptic enkephalinergic modulation underlying stereotyped behavior. P.A. Broderick, C.D. Blaha* and R.F. Lane. Dept. Psychiat., Alb. Einstein Col. Med., N.Y.10461 and Inst. of Neurosci., Univ. of Or., Eugene, Or. 97403.

We investigated the effect of an enkephalin pentapeptide analog on amphetamine-induced stereotypy in male, albino rats and the subsequent alterations both in spontaneously released dopamine and amphetamine-induced release of dopamine from the rat caudate by electrochemical methods *in vivo*.

Eight individual components of stereotyped behavior were recorded simultaneously with microswitches and counters for 4 min intervals every 20 min for 6-7 time periods, 15 min following the injection of amphetamine (2.5 mg/kg ip). The enkephalin was administered ½ hr prior to amphetamine. A separate group of animals received enkephalin alone to examine opiate-induced stereotypy. The rats, isolated in a soundproof room, were observed through an adjoining one-way mirror. The electroanalytical techniques employed were semiderivative (SDV) voltammetry and chronoamperometry. Recordings were obtained from chloral hydrate anesthetized rats, temperature controlled (37^0C) throughout each experiment. Modified working electrodes (150-175 μ), selective for dopamine, (Lane, Neurosci. Abstr. 7, 1981) were stereotaxically implanted in the anterior caudate. A Ag/AgCl reference electrode and a Pt auxiliary electrode were placed in contact with the cortex. SDV voltammograms were recorded every ten minutes at a scan rate of 10 mv sec^{-1}. Chronoamperometric measurements were made by applying a potential of 0.35 volts for 1 sec, with an interval of ½ min between measurements. Currents were proportional to dopamine concentrations *in vitro* and were used for calibration. Drugs were administered similarly to the behavioral protocol.

WY 42,896 (N-methyl tyr ser^2 ser^5 enkephalinamide) significantly inhibited all parameters of stereotypy. Enkephalin produced licking and grooming, stereotyped behaviors not usually seen with amphetamine. Dopamine release after enkephalin administration decreased 67% when compared with basal release. Further, in controlled studies conducted with amphetamine administration alone, there was a 70% increase in the dopamine signal. Pretreatment with enkephalin blocked the response normally seen with amphetamine.

These data provide evidence for (1) an inhibition of spontaneous dopamine release from the rat caudate by enkephalin (2) an inhibition of the usual dopamine releasing properties of amphetamine by the same enkephalin concomitant with an inhibition of amphetamine-induced stereotypy and (3) suggest a presynaptic site of action for the enkephalin opiates at dopaminergic nerve terminals. This is the first report of an enkephalinergic-dopaminergic interaction from direct *in vivo* electrochemical studies. (Supported by USPHS Grants MH 15788 and NS 13556).

222.10 RECEPTOR BINDING AND ANALGESIC PROPERTIES OF OXYMORPHAZONE. G. S. F. Ling*, S. Galetta* and G. W. Pasternak. (SPON: R. Price). The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Departments of Neurology and Pharmacology, Cornell University Medical College, New York, N.Y. 10021

Naloxazone, the 14-hydroxydihyromorphinone hydrazone derivative of the narcotic antagonist naloxone, has been previously shown to effectively and selectively inhibit high affinity (mu_1) sites in vitro and morphine analgesia in vivo. We now report on the actions of oxymorphazone, the hydrazone derivative of the narcotic agonist oxymorphone, on receptor binding in vitro and on analgesia in vivo. Oxymorphazone eliminates the high affinity binding component of ^{3}H-opioid binding despite extensive washes as demonstrated by both saturation studies analyzed according to Scatchard and by displacement studies using a series of ^{3}H-labeled opiates and enkephalins. Thus, oxymorphazone appears to have the same selectivity for binding studies as naloxazone. Acutely, in mice, oxymorphazone is less potent than oxymorphone (ED_{50}s of 0.8 and 0.4 mg/kg, s.c., respectively). Both quantal dose response curves were parallel. At higher doses (100 mg/kg), up to 90% of animals at 24 hours given oxymorphazone are analgesic whereas none of the oxymorphone animals are. Oxymorphazone also produces prolonged analgesia after icv administration. At a dose of 50 ug/mouse, 85% of mice treated with oxymorphazone were still analgesic 20 hours after drug administration. None of the mice treated at the same dose with oxymorphone were analgesic at this time. Other results suggest that oxymorphazone's actions are not adequately explained by pharmacokinetic differences from oxymorphone. In vivo administration of oxymorphazone at doses which produce a long-acting analgesia also inhibits the high affinity, or mu_1, site supporting the hypothesis that the drug works via prolonged receptor binding. These results strengthen the hypothesis that the mu_1 sites are important in opiate analgesia. Prolonged binding of an antagonist, naloxazone, to these sites blocks the analgesic actions of opiates, enkephalins and β-endorphin where prolonged occupation of these sites by an agonist, oxymorphazone, results in a corresponding long-acting analgesia.

223.1 SEROTONIN DISTRIBUTION IN DISCRETE REGIONS OF THE CAT HINDBRAIN. Y. Tizabi, C.H. Park* and V.J. Massari. Dept. of Pharmacology, College of Med., Howard Univ., Washington, D.C. 20059.

Although there are a few qualitative histofluorescence studies on serotonin (5HT) distribution in the cat brain, there are relatively few quantitative biochemical studies. Particularly, data on the distribution of this biogenic amine in specific individual nuclei of the hindbrain has been lacking.

Eight male mongrel cats weighing 2.5-3.5Kg were sacrificed while under light ketamine anesthesia (33mg/kg i.m.). Brains were rapidly removed, mounted on specimen plates and frozen on dry ice. Alternate serial sections of 500 µm or 50 µm thickness were cut in a cryostat at -8°C. The thin sections were stained and nuclei (N) identified. Specific brain regions were dissected from the thick sections and serotonin (5HT) concentration was determined using a specific and sensitive radioenzymatic assay.

The highest concentration of 5HT (61pG/µg Protein) was found in the dorsal raphe N. one major site of serotonergic cell bodies. High concentrations of 5HT (28-35pG/µg) were observed in the dorsal tegmental N., N. centralis superior and locus coeruleus. Moderate concentrations of 5HT (16-23pG/µg) were found in various other raphe nuclei (e.g., pontis, pallidus, obscurus), substantia nigra (zona reticulata and compacta), oculomotor N., motor N. of V and interpeduncular N. Low concentrations of 5HT (5-15pG/µg) were detected in a number of areas (e.g., ventral tegmental N., superior colliculus, central linear N., ventral tegmental area, periaqueductal grey, etc.). Very low concentrations of 5HT (1-5 pG/µg) were seen in lateral cerebellar N., N. interpositus, cochlear N., superior olivary N., etc. A comparison of the 5HT concentration in various raphe nuclei of the cat with those of the rat yields roughly comparable results except in N. raphe magnus and N. raphe obscurus where the concentration of 5HT is two times higher in the rat.

An analysis of the 5HT concentration in various noradrenergic nuclei showed increases in 5HT going from caudal to rostral, i.e., $A_6 > A_2 > A_1$. Twice as much 5HT was found in the Substantia Nigra Compacta (A9) as in the Ventral Tegmental Area (A10), two major sites of dopamine cell bodies. Thus the primary brain stem nuclei of the noradrenergic (A_6) as well as the dopaminergic (A9, A10) neuronal systems are highly innervated by 5HT terminals.

In a comparison of 21 different motor or sensory nuclei approximately 2.5 times as much 5HT was found in the motor nuclei. This may indicate a more prominent role for 5HT in motor than sensory function. This data on the relative distribution of 5HT in the hindbrain regions of the cat may be used as a reference in biochemical, pharmacological or other studies involving 5HT action or metabolism.

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223.2 ROLE OF SEROTONIN AS POSSIBLE NEUROTRANSMITTER IN THE CEREBELLAR CORTEX OF THE RAT. C. Beas-Zárate*, M.E. Sandoval* and A. Feria-Velasco (SPON: V. Alemán). Div. Developmental Biology. Unidad de Investigación Bioméd. Occidente, I.M.S.S. Guadalajara, Jal. and Centro de Investigaciones en Fisiología Celular, Universidad Nacional Autónoma de México. México, D.F., MEXICO.

Indolamine afferents to the cerebellar cortex have been described as well as the uptake of serotonin (5HT) by cerebellar slices; the role of 5HT in the cerebellar neurotransmission however is unknown. In the present work we evaluate the possible role of 5HT as neurotransmitter in the rat cerebellar cortex. We studied the Ca^{++}-dependent release of [^{3}H]5HT induced by either high K^+ concentrations or veratrine from crude cerebellar synaptosomal fractions (P_2) and cerebellar molecular layer homogenates. Results show that both molecular layer homogenates and P_2 fractions from whole cerebellum release recently accumulated [^{3}H]5HT in a CA^{++}-dependent manner. Moreover our data indicate that 5HT is basically released from nerve endings localized in the cerebellar molecular layer. Our data also suggest the presence of a high affinity, Na^+-dependent uptake of [^{14}C]5HT by P_2 fractions from cerebellum. Since both Ca^{++}-dependent release and high affinity uptake have been suggested as critical tests to identify neurotransmitters in the nervous system, our results suggest that 5HT may play a role as transmitter in the molecular layer of the rat cerebellar cortex.

This work was partially supported by Grant 790298 from CONACYT to M.E.S.

223.3 TETRAHYDROTRAZODONE (THT): SPECIFIC BINDING TO RAT BRAIN MEMBRANES. David A. Kendall*, Duncan P. Taylor, and S. J. Enna. (Spon: D. L. Temple Jr.) Department of Pharmacology and Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, P.O. Box 20708, Houston, Texas 77025 and Department of Preclinical CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Company, Evansville, Indiana 47721.

The binding of tetrahydrotrazodone (THT), a radiolabeled derivative of the antidepressant, trazodone (Desyrel®), to rat brain membranes was investigated. THT is a biologically active compound which inhibited serotonin (5-HT) transport *in vitro* and prevented conditioned avoidance responding *in vivo*. Specific binding of [^{3}H]THT (5.7 Ci/mmole) was reversible and saturable in two steps. Brain membranes exhibited two classes of sites for [^{3}H]THT, one of high affinity (K_D~10 nM) and one of low affinity (K_D~150 nM). Specific binding was highest at 4°C and between pH 7.0 and 7.2. Association and dissociation experiments revealed a dissociation constant in agreement with that obtained from ligand saturation experiments. Binding was enriched in the crude synaptic membrane component. [^{3}H]THT binding was heterogeneously distributed in the brain, being highest in cerebral cortex and the pons-medulla. A high capacity, low affinity component (K_D~1000 nM) with different pharmacologic specificity was detected in liver membranes. 5-HT was the most potent neurotransmitter in displacement experiments (K_I~10 µM). Tetrahydrotrazodone, trazodone, metergoline, clozapine, cinanserin, spiperone, and cyproheptidine were potent inhibitors of specific [^{3}H]THT binding (K_I's <100 nM). Phentolamine, MK-212, and dihydroergotoxine were notably weak in this regard. Thus, the relative potencies for displacing [^{3}H]THT binding were similar to those for inhibiting serotonin receptor binding but different from those found with respect to α_1-adrenergic receptors. Neither [^{3}H]THT nor 5-HT_2 receptor binding were significantly modified by electrolytic destruction of the raphe nucleus. Chronic imipramine treatment (10 mg/kg, i.p., 3 weeks) decreased 5-HT_2 binding but did not affect [^{3}H]THT binding. Thus [^{3}H]THT does not appear to label α_1-adrenergic or 5-HT_2 binding sites or 5-HT uptake sites. The identification of a brain-specific binding site for this analog of the clinically useful antidepressant, trazodone, may aid in better defining the mechanism of action of this drug at the molecular level.

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223.4 SELECTIVE 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT) DESTRUCTION OF THE FORNIX-FIMBRIA AND CINGULUM BUNDLE 5-HYDROXYTRYPTAMINE (5-HT) AXONS INCREASES 5-HT_1 RECEPTORS IN RAT MIDBRAIN AND SEPTUM. M. Quik and E. C. Azmitia. Depts. of Pharmacol., McGill Univ., 3655 Drummond St., Montreal, Que. H3G 1Y6 and Anatomy, Mt. Sinai Sch. of Med., N.Y., NY 10029.

Selective destruction of serotonergic fibers from the median raphe nucleus to the hippocampus decreases 5-HT uptake and in rats induces behavioral supersensitivity to administered 5-hydroxytryptophan (Azmitia et al., 1978, Nature 274, 374). It has been postulated that the biochemical basis for this behavioral supersensitivity is an increase in the number of postsynaptic 5-HT receptors in the hippocampus. On the other hand, measurement of a presumed postsynaptic 5-HT receptor (using ^{3}H-5-HT) in the hippocampus after medial raphe lesions has yielded conflicting results - no change in the receptor population (Fillion et al., 1978, Mol. Pharmac. 14, 50) and an increase in receptor number (Nelson et al. 1978, Mol. Pharmac. 14, 983). This discrepancy may have arisen because of differences in the methods used to lesion the hippocampal serotonergic system and/or possible damage to other neurotransmitter systems. Selected and localized lesions of serotonergic neurons were made by microinjection of 5,7-DHT (after pretreatment with desipramine) into the cingulum bundle and fornix-fimbria, two serotonergic hippocampal inputs from the median raphe. Two weeks later, the binding of ^{3}H-5-HT (5-HT_1 receptor) was determined in the hippocampus which receives the afferent terminals and in the striatum which receives 5-HT inputs from other tracts. In addition, the binding was measured in the septal region and the midbrain from where the fibers originates; these areas are both proximal to the site of the lesion. Scatchard analysis revealed a small but nonsignificant decline in the maximal number of binding sites (Bmax) in the hippocampus while the dissociation constant (Kd) remained the same. The striatum which receives 5-HT inputs via other 5-HT tracts was not affected by the lesion. Interestingly, a significant increase was observed in the Bmax in the midbrain (38%) and septum (12%) with no change in the Kd. These results would suggest either that ^{3}H-5-HT binding is to presynaptic 5-HT receptors or that behavioral supersensitivity to 5-HT is mediated by changes in 5-HT receptors in the midbrain and/or septal region and not the hippocampus. Experiments are currently in progress to determine the effect of lesioning on the binding of ^{3}H-spiroperidol to 5-HT_2 receptors in the same brain regions as it has been suggested that changes in behavioral supersensitivity may be related to this 5-HT receptor population.

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223.5 STIMULATION OF LATERAL HABENULA PRODUCES AN IPSP IN NEURONS OF THE RAT DORSAL RAPHE NUCLEUS. M. R. Park. Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

It is known from extracellular single-unit recordings that stimulation of the lateral habenula (LH) produces an inhibition of spontaneous action potential generation in dorsal raphe (DR) neurons (Wang and Aghajanian, Science 197:89, 1977). In the present study, the synaptic events underlying this inhibition were determined using intracellular recording techniques in rats anaesthetized with urethane (single inducing dose of 1.0 g/kg, i.p.) and ketamine (40 mg/kg, i.p., given hourly). The floor of the fourth ventricle was exposed by removing the central 3-4 mm of cerebellum. This permitted the localization of the recording site and placement of the recording micropipette by visual inspection. Intracellular recordings were made in neurons lying within 0.3 mm of the midline.

DR projection neurons, identified as such by their antidromic response to stimulation of ventral medial tegmentum (VMT), responded with an inhibitory postsynaptic potential (IPSP) to stimulation of LH. The IPSP had the character of a conductance-increase postsynaptic potential in that its amplitude was reduced during the injection of hyperpolarizing current. The latencies of the LH evoked IPSP ranged from 4 to 28 msec. Increasing the strength of the stimulating current did not produce a shift in the latency of the evoked IPSP. This latter finding is indicative of a monosynaptic lateral habenula connection to DR projection neurons. None of the units encountered responded with excitation to LH stimulation. This includes both DR projection neurons and cells belonging to the population of rapidly firing neurons which are considered to be non-serotonergic. Failure to find responses consistent with a candidate for an interneuron mediating the habenula evoked IPSP further suggests that these afferents to dorsal raphe make synaptic contacts directly upon DR projection neurons. This finding must be evaluated in light of biochemical evidence (Gottesfeld et al., Brain Res. 141:353, 1978) which has favored a polysynaptic linkage.

(Supported by NIH Grant RR05772.)

223.6 TOPOGRAPHIC DISTRIBUTION OF NEOCORTICAL PROJECTION NEURONS IN THE DORSAL AND MEDIAN RAPHE NUCLEI OF RAT. B. D. Waterhouse, J. C. Baack and D. J. Woodward. Dept. of Cell Biology, The Univ. of Texas Health Science Center, Dallas, Texas 75235.

The present study was conducted to examine the spatial organization of dorsal (DR) and median (MR) raphe neurons that project to rat cerebral cortex. Sixteen Long-Evans hooded rats (180-275 gm) received unilateral pressure injections (0.3-1.5 µl) of HRP (Miles Lab., conc. in Tris buffer) in either frontal (n=4), sensorimotor (n=7) or occipital (n=5) cortex to determine the locations of DR and MR neurons projecting to specific cortical areas. Coronal sections (40-100 µm) through the DR (B6 and B7) and MR were examined by light microscopy after carrying out the TMB reaction and staining with neutral red. The locations of retrogradely labeled cells were recorded relative to a three dimensional biological coordinate system maintained by a computer linked to a light microscope (Smith et al., Neurosci. Abst., 1981).

DR neurons labeled from cerebrocortical injections of HRP were primarily located in the ipsilateral half of the nucleus with only a few cells (less than 5%) displaced contralaterally. Neocortical projection neurons were concentrated in the middle three-fifths of the DR. Within this region of the nucleus, HRP-filled cells were distributed such that individual groups of neurons projecting to frontal, sensorimotor or occipital cortex were aligned in a partially overlapping, rostral to caudal array. Moreover, in the dorso-ventral dimension retrogradely labeled cells were clustered into three groups each corresponding to a distinct cortical region. Accordingly, DR neurons projecting to the frontal and sensorimotor areas were concentrated in the dorsal and intermediate portions of the nucleus, respectively; whereas cells projecting to the occipital region were situated most ventrally, between the medial longitudinal fasciculi. In contrast to the DR, labeling in the MR was sparse and either preferentially contralateral or bilaterally symmetrical. The zones of labeling resulting from injections confined to the neocortical gray matter overlapped with but were not coextensive with those observed following injections into 1) caudate and 2) cerebellum.

In summary, the data from the brains analyzed has delimited portions of the DR and MR nuclei which project to rat neocortex. Moreover, the results suggest that within the DR a topographic ordering exists which reflects the rostro to caudal trajectory of serotonergic axons within the cortex. This organization suggests that activity in subsets of raphe cells may independently influence separate populations of neurons within serotonergic terminal fields of the cerebral cortex. (Supported by NINCDS NS-18081, NIDA DA-02338 and the Biological Humanics Foundation).

223.7 SEROTONIN STIMULATION OF ADENYLATE CYCLASE IN ADULT GUINEA PIG HIPPOCAMPUS. A. Shenker*, S. Maayani, H. Weinstein*, and J.P. Green. Department of Pharmacology, Mt. Sinai School of Medicine of CUNY, New York, NY, 10029.

Recent attempts to classify serotonin receptors in mammalian brain have revealed an apparent multiplicity of sites. Pharmacological characterization of a receptor requires an assay in which both responses to agonists and the affinities of antagonists can be accurately measured. Serotonin-sensitive adenylate cyclase has been proposed as such an assay. We measured serotonin stimulation of adenylate cyclase activity in whole hippocampal homogenates from adult male guinea pigs. The standard assay mixture included 75 mM Tris-HCl (pH 7.4), 1 mM [^{32}P]ATP, 5 mM $MgCl_2$, 10 uM GTP, 10 uM pargyline, 0.01% ascorbate, 4 mM theophylline, 1 mM cAMP, an ATP regenerating system, and 150 ug protein. The reaction was conducted at 30°C for two minutes, during which time production of cAMP was linear.

Basal cyclase activity in this preparation was 155 ± 7 pmoles cAMP/min/mg protein (mean ± SEM, n=8). Serotonin stimulation of cyclase activity was concentration-dependent, with a Hill slope of approximately 0.7. Half-maximal stimulation (ED_{50}) was produced by 0.1 uM serotonin. Maximal stimulation of cyclase activity above basal (E_{max}) was 63 ± 3 pmoles cAMP/min/mg protein, i.e. 40% stimulation (range= 30-50%). The response to serotonin was dependent on GTP. As previously described, histamine also stimulates cyclase activity in guinea pig hippocampus; in this preparation, E_{max} was 157 ±3 pmoles cAMP/min/mg protein (100% stimulation of basal activity).

Since endogenous serotonin may modulate the response measured in this assay, experiments were designed to deplete brain serotonin content. Three pairs of guinea pigs were injected i.p. with reserpine or vehicle for 1-4 days. The ED_{50} for serotonin stimulation was not altered by this treatment. A 50% increase in net serotonin-stimulated activity was observed in each of the reserpinized animals compared to its vehicle-injected control; this increase was not attributable to a change in basal cyclase activity. In contrast, maximal stimulation of cyclase activity by histamine was unaffected by reserpine treatment. The increased response to serotonin in the adenylate cyclase assay following reserpine treatment may indicate that serotonin levels modulate the system in vivo.

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223.8 DIFFERENTIAL EFFECTS OF ZIMELIDINE AND FLUOXETINE ON CORTICAL RESPONSES TO MEDIAN RAPHÉ STIMULATION AND ON UPTAKE OF 5-HYDROXYTRYPTAMINE AND TRYPTAMINE INTO CORTICAL SLICES. J. Broadbent* and R.S.G. Jones* (Spon: J. Thornhill). Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan S7N 0X0 Canada.

Spontaneously active cortical neurones in the rat frequently respond to median raphé (RM) stimulation with a biphasic response: an early inhibition followed by excitation. It has been suggested that the early inhibition may be mediated by tryptamine (T), whereas the excitation may be a 5-hydroxytryptamine (5HT) mediated response (Jones, R.S.G. Neuropharmacology 21, in press). The effects of the uptake blockers, fluoxetine (F) and zimelidine (Z), on this evoked cortical response to RM stimulation were investigated. Stimulation of the RM (single pulse, monophasic square waves, 2 msec duration, 50-300 µA, every 3-4 secs) was used to evoke responses in the fronto-parietal cortex. Responses were recorded with NaCl (3M) filled microelectrodes. Peri-stimulus histograms were constructed after control responses were established and Z or F (up to 10 mg/kg) was then injected into the tail vein of the rat. Following F administration, consistent enhancement of both the inhibition and excitation to RM stimulation occurred. Z, however, potentiated the excitatory response without altering the early inhibition. The effects of F and Z on the simultaneous uptake of T and 5HT into fronto-parietal cortical slices were also investigated. Cortical slices from rats sacrificed 20 minutes after tail vein injections of Z or F (5 or 10 mg/kg) were incubated with ^{14}C-5HT and ^{3}H-T for 10 min at 37°C. Radioactivity in slices was measured by liquid scintillation counting after separation from the incubation medium by filtration. Diffusion effects were omitted by running blanks at 0°C and subtracting the count from that of the samples. F and Z exerted differential effects on the uptake of the two amines. F reduced both 5HT and T uptake, however, Z only reduced the uptake of 5HT. The present data demonstrate that F and Z have differential effects on biphasic responses to RM stimulation in vivo and on the simultaneous uptake of 5HT and T in vitro. These data together with other recent results (Jones, R.S.G. and Broadbent, J., Neuropharmacol., 21, in press) provide supporting evidence for the hypothesis that T may mediate early inhibition to RM stimulation and 5HT may mediate excitation.

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223.9 A DUAL AND DIFFERENTIAL EFFECT OF ASCORBATE ON [^{3}H]SEROTONIN AND [^{3}H]SPIPERONE BINDING IN RAT CORTICAL MEMBRANES.
S.F. Muakkassah-Kelly*, J.W. Andresen*, J.C. Shih and P. Hochstein*.
Institute for Toxicology, School of Pharmacy and Department of Biochemistry, School of Medicine, University of Southern California, Los Angeles, California 90033.

We have previously demonstrated that ascorbate induced lipid peroxidation in rat cortical membranes results in irreversible decreases in the number of binding sites of both [^{3}H]serotonin ([^{3}H]5-HT) and [^{3}H]spiperone. This decrease was linearly correlated to the amount of malonyldialdehyde (MDA) produced (an index of lipid peroxidation) (BBRC, 104:1083, 1982).

Since most commonly used binding assays (Peroutka, S.J. and Snyder, S.H. Mol. Pharm. 16:687, 1979) contain 5.7 mM ascorbate, 4 mM $CaCl_2$ in 50 mM Tris buffer, pH 7.4, we investigated the effect of the assay constituents on lipid peroxidation and on [^{3}H]5-HT and [^{3}H]spiperone binding. In the absence of $CaCl_2$, ascorbate (.057-5.7 mM) caused increased MDA formation. 4.0 mM $CaCl_2$ had no effect on MDA production in the presence of 0.057-0.57 mM ascorbate. However, under the standard assay condition, (5.7 mM ascorbate), 4 mM $CaCl_2$ inhibited MDA production by 85%, whereas the inactivation of [^{3}H]5-HT binding was inhibited only by 50% indicating an additional effect of ascorbate which is independent of lipid peroxidation. This additional effect of ascorbate was detected in the presence of EDTA (an inhibitor of lipid peroxidation) and was reversible after washing of the membranes. Scatchard analysis of the direct effect of ascorbate revealed decreases in both Kd and Bmax of [^{3}H]5-HT. This direct effect of ascorbate may be related to its reducing properties, as other reducing agents (dithiothrietol, glutathione, Na metabisufite) also decreased the specific binding of [^{3}H]5-HT. Thus, our results show that ascorbate has a dual effect on serotonin binding. Ascorbate induced peroxidation causes an irreversible decrease in Bmax but not Kd of [^{3}H]5-HT. Secondly, independent of lipid peroxidation, ascorbate causes decreases in both Kd and Bmax of [^{3}H]5-HT binding.

In general, [^{3}H]spiperone is considered to label another type of serotonin receptor in the cortex. Similar to [^{3}H]5-HT, [^{3}H]spiperone binding was decreased as a result of ascorbate induced peroxidation. However, in contrast to [^{3}H]5-HT, [^{3}H]spiperone binding was not directly affected by ascorbate or any of the reducing agents studied. These results indicate a difference in the biochemical nature of the two serotonin binding sites.

223.10 METABOLIC REGULATION OF SEROTONIN RELEASE FROM DEPOLARIZED HIPPOCAMPAL SLICES. Sid Auerbach* and Peter Lipton. (SPON: H.J. Karavolas). Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

We have developed a kinetic model to analyze the time course of serotonin release from depolarized hippocampal slices. The model assumes that 5-HT release from a releasable pool follows first order kinetics and that supply of 5-HT to this pool depends on its metabolism. 5-HT release is expressed as a function of time after onset of depolarization:

$$R_t = (S_{ro} - K_s/K_r)(1-e^{-K_r t}) + K_s t \quad - \quad (1)$$

Rt = total 5-HT released at time = t; S_{ro} = 5-HT initially present in the releasable pool; K_r = rate constant for release from this pool, and should increase as exocytosis is activated by increased cytosolic Ca^{++}. K_s = amount of 5-HT transferred into the releasable pool per min and should increase when 5-HT availability is increased by increasing synthesis or by decreasing breakdown. The equation predicts that altering K_r will affect early 5-HT release and that altering K_s will have major effects during prolonged release of 5-HT. We tested these predictions.

Rat hippocampal slices were incubated in oxygenated buffer for 60 min and then transferred to buffer containing elevated 5-HT. The buffer was sampled frequently and analyzed for 5-HT by HPLC. Release curves were analyzed by nonlinear regression to determine the closest fit to (1) and so to determine K_r, K_s and S_{ro}. The release curves were well fit by equation (1).

Reducing buffer Ca^{++} decreased 5-HT release during the first 9 min of depolarization in high K^+ buffer but did not affect the rate of release during the final 31 min of incubation. K_r, the release constant, was decreased from .22 ± .02 min^{-1} to .12 ± .02 min^{-1}. K_s was unaffected.

When buffer tryptophan was reduced from 2 µM to 0 µM, 5-HT synthesis during depolarization was decreased 65%. 5-HT release was unaffected during the first 5 min but release between 5 and 40 min was reduced by 50%. K_s was decreased from .049 ± .008 to .024 ± 0.001 ng/mg prot/min. When 5-HT breakdown was blocked with pargyline there was no increase in release during the first 5 min of depolarization but between 5 and 40 min, release increased by over 100%. K_r was unaffected and K_s was increased from .040 ± .004 to .160 ± .007 ng/mg prot/min.

Thus 5-HT release during prolonged depolarization is strongly dependent on 5-HT metabolism. The results indicate that 5-HT release occurs from a pool which contains about 20% of total tissue 5-HT and that the rate of release is proportional to the amount of 5-HT present in this small pool. The size of this pool is strongly dependent on the rates of 5-HT synthesis and breakdown during depolarization.

223.11 EFFECTS OF ZINC CHELATION BY DITHIZONE ON HIPPOCAMPAL SEROTONIN. T. DeNeal, M.E. Trulson, G.A. Howell, and C.J. Frederickson. Laboratory for Neurobiology, Univ. of Texas at Dallas, Richardson, Tx. 75080.

One approach to the study of the role of mossy-fiber zinc is to manipulate the zinc and search for resulting changes in the hippocampus. When the chelator, dithizone, is administered, it reaches the hippocampus and binds accessible zinc, as evidenced by the zinc dithizonate which appears in the mossy fiber region. Zinc chelation by dithizone previously has been shown to alter the mossy fiber-to-pyramid evoked potential (Crawford, et al. Pharmacologist 1973 15:197) and alter mossy-fiber terminal ultrastructure (Otsuka et al. Acta Histochem. Cytochem. 1975 8:91). Here we report that chelation with dithizone also selectively reduces the level of serotonin (5-HT) in the hippocampus.

Twenty-eight adult, male, albino rats received dithizone solution (0.5 gm of dithizone, 20 drops NH_4OH, 100 ml H_2O, 5 ml ethanol; 30ml/kg, i.p.) and 32 control animals received vehicle only. Thirty min after injection animals were decapitated, brains were removed, hippocampi and overlying neocortex were removed, and frozen in liquid N_2. 5-HT and 5-HIAA were assayed spectrofluorimetrically after isolation by solvent extraction.

Compared to controls, 5-HT in the hippocampi of the dithizone animals was reduced by 13% ($p < .001$, 2-tailed t test) whereas cortical levels of 5-HT were unaffected (dithizone group = 99% of control, $p > .5$). Hippocampal levels of 5-HIAA were not significantly different for the two groups (dithizone = 101% of control; $p > .5$); cortical 5HIAA, however, was elevated (+15%) in the dithizone group ($p < .001$).

A serendipidous finding was a relationship between the apparent age (i.e. weight) of animals and the effects of dithizone. Specifically, for the "old" animals (450-650 gm; N=14 for dithizone, 16, control) the decrease in hippocampal 5-HT was much larger (-19%, $p < .001$) than for the "young" (300-449 gm) animals (-6%, $p < .2$). Conversely, the increase in cortical 5-HIAA was larger for the young animals (+21%, $p < .002$) than for the old animals (+12%, $p < .1$).

The possible role of ageing notwithstanding, the present results indicate that dithizone chelation selectively depletes hippocampal 5-HT while simultaneously increasing neocortical (but not hippocampal) 5-HIAA. One interpretation of the results might be that chelation increases firing rates of serotonergic afferents to hippocampus and cortex alike, yielding an increase in cortical 5-HIAA, but also causes selective depression of hippocampal 5-HT synthesis, thus depleting hippocampal 5-HT without a concomitant increase in hippocampal 5-HIAA.

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223.12 THE DISTRIBUTION OF HYPOTHALAMIC HISTAMINERGIC NEURONS: AN IMMUNOHISTOCHEMICAL STUDY. B.J. Wilcox and V.S. Seybold. Department of Anatomy, University of Minnesota Medical School, Minneapolis, MN 55455.

The existence of histaminergic cell bodies within the hypothalamus has been suggested by biochemical evidence. However, their exact location has not been determined anatomically. As an extension of previously reported work (Wilcox and Seybold, Neuroscience Lett. '82) the present study employs immunohistochemical techniques to visualize cell bodies displaying histamine-like immunoreactivity in order to map their distribution.

Fifty µm serial sections of hypothalamus from female rats treated with intraventricular colchicine (50µg) were cut on a freezing microtome. Alternate sections were immunostained by the peroxidase-anti-peroxidase method of Sternberger. Adjacent sections were processed as absorption controls. Primary antiserum generated in guinea pigs against histamine was used at a dilution of 1/1000 in phosphate buffered saline with 0.3% Triton X-100. The secondary and tertiary antisera (goat-anti-guinea pig and guinea pig PAP, respectively) were both used at a dilution of 1/100 in phosphate buffered saline with 0.3% Triton X-100. Camera lucida drawings were made of each tissue section, and the distribution of cell bodies exhibiting histamine-like immunoreactivity was recorded.

Immunostained cell bodies were found in the hypothalamus at the dorsal tip of the third ventricle beginning approximately 5mm anterior to the frontal zero plane according to Konig and Klippel (A 5150µ). As the cell formation extends caudally, it expands laterally. At the level of the mid-median eminence (approximately A 4380µ), the group of cell bodies was seen at its largest extent stretching across the lateral hypothalamus reaching the optic tracts. The caudal extent of the cell formation was approximately A 3750µ. The cell bodies were oriented mainly in a medio-lateral direction. Adjacent sections processed as absorption controls showed no immunostained cell bodies.

The distribution of immunostained cell bodies was scattered through portions of nucleus paraventricularis, nucleus dorsomedialis, and nucleus lateralis hypothalami. Since these nuclei have known projections to some brainstem nuclei and telencephalic structures, it is possible that the histaminergic neurons identified in this study are responsible for the neuronal histamine content of these areas. With the location of the hypothalamic histaminergic cell bodies established, further studies to determine their projections can be pursued.

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223.13 ^{3}H-TRYPTAMINE: CHARACTERIZATION OF BINDING SITES IN RAT BRAIN
C. S. Cascio* and K. J. Kellar (Spon. K.L.Dretchen)
Department of Pharmacology, Georgetown University School of Medicine and Dentistry, Washington, D.C. 20007

Tryptamine is formed in brain, evokes electrophysiological responses and is behaviorally active. We have recently reported that ^{3}H-tryptamine (New England Nuclear, 24-31 Ci/mmol) binds to rat cerebral cortex (Europ. J. Pharmacol. 78: 475, 1982). The binding of ^{3}H-tryptamine is rapid ($t_{1/2}$ association≈12 min), reversible ($t_{1/2}$ dissociation ≈ 7 min) and of high affinity (K_d ≈ 3nM, Bmax ≈ 25 pmol/g tissue). Hill plots have a coefficient of ~ 1, indicating a lack of cooperativity and that ^{3}H-tryptamine binds to a single class of sites. Subcellular distribution studies indicate that the binding site is concentrated in the synaptosomal and mitochondrial fractions.
In brain region distribution studies, binding is highest in the striatum, hippocampus and cortex, and lowest in the spinal cord and pons-medulla. The binding is decreased by monovalent cations (IC_{50}: NaCl≈49 mM , KCl≈30 mM, LiCl≈25 mM) but relatively insensitive to $MgCl_2$ and $CaCl_2$ (IC_{50} >100 mM). GTP-tris salt decreases the binding (IC_{50}≈10 uM), while ATP-tris salt has no effect. In drug competition studies, 5-HT is moderately potent in displacing binding of ^{3}H-tryptamine (K_I≈280 nM); however, LSD, methysergide, mianserin and spiperone are quite weak (K_I>50 uM). Dopamine is also moderately potent (K_I≈470 nM); however, apomorphine and butaclamol are weak. Of the methyl and dimethyl tryptamines examined, 5-methyl tryptamine is the most potent of the drugs (K_I≈20 nM). Other potent competitors for the ^{3}H-tryptamine binding site include: tetrahydro-β-carboline, phenylethylamine and amphetamine. ^{3}H-tryptamine binds to a site which appears to be distinct from serotonin sites. The binding site appears to have a great deal of specificity for tryptamine and may mediate physiologic actions of tryptamine in brain.

223.14 IN VITRO AUTORADIOGRAPHIC LOCALIZATION OF [^{3}H]TRYPTAMINE BINDING SITES IN RAT BRAIN.
D.C. Perry, D.C. Manning and S.H. Snyder. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Tryptamine, a trace amine found in the CNS, exhibits behavioral and electrophysiological effects which suggest a role as a central neurotransmitter. It elicits primarily depressant postsynaptic effects when applied iontophoretically to cortical neurons, in contrast to the predominantly excitatory effects seen with serotonin (5-hydroxytryptamine) on the same neurons. Recently, Kellar and Cascio (Eur. J. Pharmacol., 78:475, 1982) reported specific high affinity binding of [^{3}H]tryptamine in rat brain homogenates.

To further clarify the role of tryptamine in the brain, we have studied [^{3}H]tryptamine binding to rat brain tissue slices by in vitro autoradiography. Preliminary results indicate that binding in 10 μ brain sections is similar to that seen in homogenates. [^{3}H]Tryptamine binds to a single class of sites with an affinity of 3 nM and 86% specific binding under optimal conditions (50 mM Tris HCl, pH 7.5, 5 mM ascorbic acid, 20 μM pargyline, 45 min incubation @ 4°C). Highest binding is seen in striatum, hippocampus and cortex; detailed microscopic localization will be presented.

223.15 DISTRIBUTION OF FREE AND TOTAL PHENYLETHYLAMINE IN WHOLE BLOOD AND URINE FROM NORMAL HUMAN SUBJECTS.
P. A. Shea, K. S. Moore*, S. Wade*, S. R. Dunlop* and H. C. Hendrie*. Inst. Psychiat. Res., Dept. of Psychiatry and Biochemistry, Indiana Univ. Sch. of Med. Indianapolis, IN 46223.

Phenylethylamine (PEA) is a trace catecholamine found in the CNS as well as other tissues and has been reported to be involved in the pathophysiology of the major psychiatric disorders. Evidence for this has been primarily based on comparisons between urinary levels of PEA between patients and normals. Conflicting results, however, have been reported and may be caused in part by variations in the specificity and sensitivity of the assays for PEA. Another source of variation is urine itself. The clearance and urinary accumulation of PEA may be dependent on urinary pH, volume, creatinine, and patient compliance for completion of a 24 hr. collection. Therefore, using the specific and sensitive method of gas chromatography-mass spectrometry we have studied the distribution of both free and conjugated PEA in whole blood from normal subects so that in future studies comparisons between psychiatric patients and controls might be less variable than urinary levels. Other measurements include urinary pH, volume, creatinine, free and conjugated PEA and whole blood MAO activity. Blood was drawn during the morning hours from 17 control subjects into evacuated tubes containing EDTA and pargyline. Twelve hour urine collections were initiated 8 to 10 hrs. before blood draw. The levels of free and total PEA in whole blood are 0.15 ± .09 (S.D.) and 0.26 ± 14 ng/ml, respectively. In platelet free plasma unconjugated PEA represented 8% of whole blood total PEA while conjugated PEA was 31%. Platelet rich plasma had a similar percentage. Therefore, the majority of whole blood total PEA, (61%), was associated with the red blood cell (RBC) fraction. Of the 39% found in the plasma 81% is in the conjugated form whereas 20% is conjugated in the RBC fraction. Whole blood total PEA was the only fraction found to correlate significantly with total plasma PEA ($p < 0.001$). There were no significant correlations of MAO activities with PEA in any blood fractions. Urinary values for PEA were 6.8 ± 3.8 and 3.8 ± 2.1 ng/ml for total and free, respectively. Of the possible factors associated with urinary PEA levels, only whole blood MAO activities correlated significantly with total urinary PEA ($p < .02$). Inter and intra patient variation, daily blood variation and other parameters concerning the distribution and measurement of PEA in normals will be presented. (Research supported in part by a grant from the Indiana Deptartment of Mental Health 178-679-005).

223.16 THE EFFECTS OF ANTIBODY FOR MELATONIN ON CORTICAL EEG IN THE RAT.
M. E. Pierce*, J. H. Peck, L. J. Grota and G. M. Brown. Dept. of Psychology, Ithaca College, Ithaca, NY 14850.

Melatonin has been shown to have pronounced effects on various endocrine functions and on activity of the central nervous system. Melatonin has been shown to have certain electrophysiological effects. Injections of melatonin intraperitoneally in chickens and to the preoptic region in cats resulted in desynchronization of the EEG and sleep. The effects of melatonin on sleep have also been studied in humans with changes occurring in GSR, heart rate and number of REM episodes (Anton-Tay, F., Adv. Biochem. Psychopharm., 11, 1974; Izumi, K., et al. Canadian J. Physiol. 51, 1973). Although injections of melatonin in cats do not alter induced epileptiform activity in cortical areas and limbic structures, sensory evoked activation of the spiking activity from primary sensory areas was reduced (Fariello, et al. Neurosci. News letters, 3, 1976). Intravenous injections of melatonin have been shown to cause improvement in symptoms and in EEG records for temporal lobe epilepsy patients (Anton-Tay, F., et al. Life Science, 10, 1971). Development of an antibody for melatonin made it possible to block melatonin (Grota, L., et al. Canadian J. Biochem., 52, 1974). Intraventricular injections of antimelatonin antibody has been reported to cause an increase in cortical epileptiform activity (Fariello, et al. Neurology, 27, 1977). The purpose of the present study was to confirm and extend these findings.

Male Charles River (CD) rats, 90-120 days of age, were anesthetized with sodium pentobarbital and had a cannula stereotaxically placed in the lateral ventricle. Surface electrodes were placed ipsilaterally, anterior and posterior to the cannula. Following a baseline recording, 5 uL. of either antimelatonin antibody or normal saline was injected into the lateral ventricle, and the EEG record was examined for epileptic spiking. Unfortunately, recording under pentobarbital anesthesia was not satisfactory due to large amounts of spiking in the baseline record. Attempts to remedy this condition by using different electrode configurations all failed and we were unable to replicate the data collected previously.

In order to get a more satisfactory baseline EEG, we changed from pentobarbital to urethane anesthesia. While there are as yet unexplained fluctuations in the baseline EEG under urethane anesthesia, it does appear that the antimelatonin antibody does produce spiking activity in the EEG record.

223.17 DAILY RHYTHMS IN ACTIVITY AND MELATONIN SECRETION AMONG RATS IN A NATURALISTIC ENVIRONMENT. H.J. Lynch*, M.H. Deng*, P. Ronsheim*, and R.J. Wurtman. (SPON: L. Young). Laboratory of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139.

Daily rhythms in activity and melatonin secretion were studied among rats housed in cages equipped with dark burrows. While the interiors of the burrows were continuously dark (<0.013 $\mu W/cm^2$), externally ambient lighting consisted of alternating 12-hour periods of daylight (66 $\mu W/cm^2$; 0700-1900) and night light (0.030 $\mu W/cm^2$; 1900-0700). Activity was assessed in terms of water consumption per 12-hour period and by electronic recording of animals entering and leaving their burrows. Rhythmic melatonin secretion was inferred from measurements of pineal melatonin content at mid-daylight period or at mid-night light period. When animals had free access to dark burrows, activity and food and water consumption were concentrated in the night light portion of their day; they remained quietly in their dark burrows during the daylight period, emerging only briefly and infrequently into the lit environment. Pineal melatonin content was low (0.45±0.07 ng/gland) in all animals killed at mid day (1300 h) when they were inactive and occupying their dark burrows. In contrast, pineal melatonin levels were elevated (1.80±0.45 ng/gland) in all animals killed at mid night (0100 h) even though they were active and exposing themselves to light (0.030 $\mu W/cm^2$). If the doors to the dark burrows were closed at 0800 (one hour after the onset of day light) and opened at 1900, after the onset of night light, activity and food and water consumption were restricted to 1900-0800. In these circumstances, pineal melatonin content was not elevated, neither at mid day (0.33±.04 ng/gland) nor at mid night (0.36±0.05 ng/gland). However, when access to the burrow was kept open, and food and water were made available both inside and outside of the burrow, activity and melatonin secretion remained associated with the night light period. These observations suggest that in a naturalistic artificial environment, rats with access to dark burrows exhibit phase-locked rhythmic patterns of activity and melatonin secretion such that peak values occur when the animals expose themselves to light (during the night light period), and minimal values occur when the animals choose to remain under less light, i.e., in dark burrows (during the daylight period).

224.1 LECTIN CYTOCHEMISTRY OF INTRACELLULAR MEMBRANES SHOWING HETEROGENEITY OF SACCULES OF THE GOLGI APPARATUS AND HETEROGENEITY OF MITOCHONDRIAL MEMBRANES IN THE CHICK CILIARY GANGLION.
J.P. Tremblay and E. Philippe*, Laboratoire de Neurobiologie, Dept. d'Anatomie, Université Laval, Québec, Canada. G1K 7P4.

In the nervous system, the presence of glycoproteins as membrane constituants is generally well accepted. Their distribution in the plasma membrane has been investigated by several techniques including the utilization of various lectins. These lectins have the ability to bind specifically to sugars present in glycoproteins and glycolipids. In this study, which is part of a larger investigation of the recycling of membranes in the chick ciliary ganglion, slices of ciliary ganglia were incubated with one of the following lectins coupled with peroxidase: SBA (Soybean agglutinin) and DBA (Dilochos biflorus agglutinin). Both lectins which are inhibited by the same sugars (α-N-acetyl-D-galactosamine and D-galactose) label however different intracellular organelles. In controls, which are pre-incubated with these sugars, and coincubated with sugars and a lectin-peroxidase, no labelling was observed. An identical result was obtained with controls pre-incubated with a crude lectin and incubated with a lectin-peroxidase. The SBA-peroxidase was especially seen within postsynaptic ciliary cells in saccules of cis (forming) face of the Golgi apparatus. No labelling was observed in saccules of the trans (maturing) face. To our knowledge, this is the first time that specific labelling of one saccule of the cis face of the Golgi apparatus is obtained. Small areas at one end of the cisternae of the rough endoplasmic reticulum are also occasionally clearly labelled. Labelling is also clearly seen in cytoplasmic membrane exposed to the extracellular space. In Schwann cells, only Ω-like profiles were labelled. In contrast, the DBA-peroxidase labels presynaptic elements in the synaptic caliciform endings and preganglionic axons. In these areas the marker was seen on the outer mitochondrial membrane (OMM), on some vacuoles, on smooth endoplasmic reticulum, on some synaptic vesicles and on neurofilaments. No organelles were labelled by DBA-peroxidase in ciliary and Schwann cells. Morphological and physiological relationships between these organelles have already been described. Droz et al. (Brain Res. 93, 1-13, 1975) have shown that mitochondria were juxtaposed to the SER. Spacek and Lieberman (J. Cell Sci. 46, 129-147, 1980) have also shown that the OMM as in fact a part of SER. Other authors have suggested that synaptic vesicles originate either from the OMM or SER network. It is interesting that although these two lectins are inhibited by the same sugars, they do not label the same organelles. This specificity of labelling may be due not only to the presence of one of these specific sugars but also to their position in the sugar chain. (Supp. by the MRC).

224.2 EVALUATION OF WHEAT GERM AGGLUTININ IMMUNOHISTOCHEMISTRY AS A NEUROANATOMICAL METHOD FOR RETROGRADE, ANTEROGRADE, AND ANTEROGRADE TRANSSYNAPTIC LABELLING IN THE CAT VISUAL AND OCULOMOTOR SYSTEMS.
R.F. Spencer, H. Baker, and R. Baker. Dept. Anat., Med. Coll. of Va., Richmond, VA 23298; Dept. Neurol., Cornell Univ. Med. Ctr., New York, NY 10021; Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Recent studies have demonstrated that the lectin wheat germ agglutinin (WGA) is a sensitive marker for studies of neuronal connectivity. WGA is incorporated by neurones and/or axon terminals and is axonally transported in anterograde or retrograde fashion. The uptake and transport of WGA is apparently dependent upon selective binding to intact neuronal surface membranes. Intraaxonal injection of WGA into physiologically-identified second-order vestibular axons results in immediate binding and failure of transport to either terminal arborizations in the oculomotor nucleus or neuronal somata in the vestibular nucleus. Furthermore, WGA injected intraocularly labels neurones in the lateral geniculate nucleus (LGN), pretectal nucleus of optic tract (NOT), and superior colliculus (SC). The present study extends our previous light microscopic findings with electron microscopic observations of retrograde and anterograde transport of WGA as demonstrated by the indirect peroxidase-anti-peroxidase immunohistochemical procedure.

Injection of WGA into extraocular muscles resulted in granular retrograde labelling of motoneurones (MN) in the abducens nucleus or appropriate subdivisions of the oculomotor nucleus. Reaction product was associated with tubules of agranular reticulum and secondary lysosomes within MN somata and proximal dendrites. There was no evidence of transneuronal labelling to synaptic endings on the MN soma-dendritic surface within the post-injection survival periods (24-72 h) of this study.

Intraocular injection of WGA resulted in anterograde terminal labelling at 24 and 36 h in LGN and SC. Flocculent reaction product was associated with all membrane components (e.g., synaptic vesicles, mitochondria, agranular reticulum, axonal inner surface membrane) within synaptic endings in LGN and SC. After 48h, WGA labelling was observed within both pre- and postsynaptic profiles, and by 72 h labelling was predominantly postsynaptic. There was no evidence of reaction product in the extracellular space or in association with neuroglial profiles. These findings suggest that the postsynaptic labelling of neurones in LGN and SC is the result of a specific release mechanism that presumably is confined to the synaptic contact zone and possibly is related to the turnover of synaptic vesicle membranes at that site.

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224.3 NEUROANATOMICAL AND BIOCHEMICAL STUDIES OF A RADIOLABELED DERIVATIVE OF WHEAT GERM AGGLUTININ.
D. A. Steindler*, R. H. Bradley, H. Imai* and B. K. Trosko*. (SPON: R. A. Pax). Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Retrograde and anterograde axonal transport of wheat germ agglutinin (WGA) has been described in neuroanatomical studies using either autoradiographic, histochemical or immunocytochemical methods of detection. In addition to its use with other tracer substances for determining the existence of collateralized pathways, descriptions of a superior sensitivity of WGA compared to, for example, horseradish peroxidase warrant its use in examining fine aspects of interneuronal connectivity. In the present study, the bidirectional axonal transport of affinity-purified WGA, N-[acetyl-^{3}H] (New England Nuclear) has been autoradiographically demonstrated in the appropriate structures following pressure or iontophoretic injections in mainly the cerebrum or cerebellum of adult mice and rats (survival times 16 hr-7 days). In addition to employing a recently developed procedure for the rapid detection of [^{3}H]acetyl-WGA injection sites, projection sites were processed for light microscopic autoradiography on frozen or vibratome sectioned material (exposure times 1-15 wks) following formaldehyde-glutaraldehyde fixation. After cortical injections of [^{3}H]-acetyl-WGA (S.A. 0.325-0.197 mCi/mg; 0.16-0.8% protein w/v), the patterns of anterograde and retrograde labeling observed in various thalamic, monoaminergic, and precerebellar nuclei was similar to that seen following similarly placed HRP injections. The most striking difference between injections of [^{3}H]acetyl-WGA and HRP is the ability to produce extremely small injection sites with the lectin that generate relatively large amounts of autoradiographic bidirectional axonal labeling. In addition, there is evidence from subcortical white matter injections of the lectin that suggests this tracer might not be taken up axons of passage in the CNS. High specific activities in addition to the sensitivity of [^{3}H]acetyl-WGA axonal tracing allows the use of short exposure times (e.g. 1-2 wks) that still lead to an extensive signal over retrograde labeled neurons. Finally, this radiolabeled lectin preparation has been analyzed using two sodium dodecyl sulfate polyacrylamide gel electrophoresis systems. Findings from Coomassie blue stained gels, fluorography and scintillation counting of fresh gels indicate the existence of predominantly monomeric as well as dimeric forms of the lectin (approximately 18,000 and 36,000 daltons, respectively), with the presence of little or no banding above or below these values. This highly-purified derivatized WGA preparation, which is transported in both anterograde and retrograde directions by neurons, is thus extremely valuable for solving hodological as well as certain cytological issues within the CNS.
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224.4 RATES OF ANTEROGRADE AND RETROGRADE AXONAL TRANSPORT OF WHEAT GERM AGGLUTININ IN THE VISUAL PATHWAY OF THE EMBRYONIC CHICK.
Taichang Jang* and W.J. Crossland (SPON: J.A. Rafols). Dept. of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201.

The lectin wheat germ agglutinin conjugated to horseradish peroxidase (WGAHRP) is a valuable tool for the study of neuronal connections in adult and embryonic nervous systems. WGAHRP has a high affinity for N-acetylglucosamine and sialic acid. However, little is known about the rate of axoplasmic transport of WGAHRP.

Five-microliter injections of a 1% aqueous solution of WGAHRP (Sigma, L-2384) were made into the vitreous bodies of White Leghorn chick embryos on the twelfth or thirteenth day of incubation (stage 36-37). According to our measurements, the transport distance from a point near the anterior pole of the retina to the caudo-dorso-medial pole of the tectum is 21.5 mm. After survival periods of 1½ to 6 hours the embryos were sacrificed, the brains sectioned, and the sections reacted with tetramethylbenzidine.

The reaction product was found in the caudo-dorso-medial tectal pole as early as two hours after injection. Disregarding the time for uptake of the lectin by the retinal ganglion cells, the rate of anterograde axoplasmic transport is in excess of 240 mm/day. Furthermore, observations of the isthmo-optic nucleus, which sends axons to the retina, revealed light retrograde labeling of cell bodies in the lateral portion of the nucleus at 2½ hours survival. Since this region of the nucleus sends axons to the anterior pole of the retina, the estimate of the isthmo-optic tract length is 17 mm, yielding a retrograde transport rate of at least 160 mm/day.

Thus the anterograde transport rate of WGAHRP is consistent with that known for the rapid transport of proteins and glycoproteins in the vertebrate nervous system. The rate of retrograde transport is at least as great as that demonstrated for horseradish peroxidase alone in the post-hatch chick.

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224.5 DIFFERENTIAL CNS TRANSPORT OF LECTIN-PEROXIDASE CONJUGATES. R.G. Wiley, W.T. Talman and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We sought to determine: (1) if there are differences in CNS transport among lectins, perhaps related to their oligosaccharide binding specificities, and (2) how lectin transport by CNS neurons might differ from peripheral neurons. Horseradish peroxidase (HRP)-lectin conjugates were microinjected (1 mg/ml; 200 nl) into the caudate nucleus of rats under halothane anesthesia. After 24-48 hr. animals were reanesthetized with pentobarbitol and perfused transcardially with aldehyde fixative followed by 15-30% sucrose solution. Brains were removed, sectioned at 40 μ on a freezing microtome, and every third section from the injection site to the rostral pons was mounted on slides. HRP was demonstrated with tetramethylbenzidine and H_2O_2. Darkfield examination revealed anterogradely labeled neuropil in the globus pallidus and pars reticulata of the substantia nigra (SN), and retrogradely labeled cell bodies in the pars compacta of SN, intralaminar nuclei of the thalamus, cingulate cortex and dorsal raphe. Three patterns of transport were observed: (1) wheat germ agglutinin (WGA), cytisus sessifolius (CSA) and ulex europeus-II (UEA-II) were transported both antero- and retrogradely; (2) bandeirea simplicifolia-II (BSA-II) and solanum tuberosum (STA) were preferentially transported in the anterograde direction; (3) conjugates of ricinus communis-I and -II, arachus hypogea, ulex europeus-I, dolichos biflorus, glycine max and wistaria floribunda were not reliably transported. Controls to demonstrate that specific binding was responsible for the observed transport included: (1) coinjection of comparable amounts of free HRP and unconjugated lectin which did not produce significant labeling; (2) coinjection of lectin-HRP conjugate with a 100-fold excess of free lectin which significantly reduced labeling; (3) coinjection of WGA-HRP or UEA-II-HRP with 40 mg/ml N,N',N''-triacetylchitotriose which did not reduce labeling (presumably due to dissociation of lectin from oligosaccharide inhibitor in vivo). To compare lectin transport by central and peripheral neurons directly, ricinus communis-II-HRP was injected into the nucleus of the tractus solitarius (NTS), the terminal field of vagal sensory neurons of the nodose ganglion. Nodose ganglion neurons were selectively labeled and killed, but none of the CNS neurons afferent to the NTS were labeled or killed. We conclude: (1) lectins binding to oligomers of N-acetylglucosamine (WGA,CSA, UEA-II, BSA-II, STA) are more reliably transported by central neurons than lectins of other binding specificities; (2) ricinus communis-II appears to differentiate between central and peripheral neurons. Differences in CNS lectin transport may reflect the relative abundance of specific oligosaccharide sequences in certain glycoproteins on the external surface of central neurons.

(Supported by grant HL 18974)

224.6 IMMUNOHISTOCHEMICAL LOCALIZATION OF AXONALLY TRANSPORTED PHA-L LECTIN TO DEMONSTRATE THE FINE MORPHOLOGICAL DETAILS OF EFFERENT CONNECTIONS IN THE CNS. C.R. Gerfen and P.E. Sawchenko. The Salk Institute, La Jolla, CA 92037.

We have examined the potential use of axonally transported phaseolus vulgaris-leucoagglutinin (PHA-L), a lectin from the red kidney bean, as an anterograde marker for tracing connections in the CNS. The PHA-L is injected iontophoretically, and, after an appropriate survival time (between 3 and 14 days in the systems we have studied in the rat brain) the tissue is processed by a standard immunohistochemical procedure, using either fluorescent or horseradish peroxidase labeling. This approach has been found to have several advantages over other anterograde labeling methods. Included among these are: (1) the injection sites can be limited to 100-200 μm in diameter and are clearly demarcated by the fact that neurons within these sites are completely filled with the marker. (2) The morphology of the labeled neurons in the injection site is usually clearly demonstrated: in addition to the axons, the cell somata, the dendrites and even dendritic spines are clearly labeled. (3) The fine morphological details of efferent axons and their terminals are recognizable, and in this respect the method gives results comparable to those in Golgi preparations. One can readily see fine collateral branches, boutons en passant, and various terminal specializations. Anterograde (as well as retrograde) transport of other lectins, such as wheat germ agglutinin (WGA), is clearly demonstrable using immunohistochemical techniques (Lechan et al., J. Histochem. Cytochem., 29:1255-1262, 1981), but WGA does not provide the morphological detail obtainable using PHA-L. (4) In every system we have examined, with even the most restricted injections, and regardless of the length of the projection, the pattern of axonal ramifications and their terminal specializations are exquisitely revealed. (5) The PHA-L lectin, in marked contrast to WGA, seems to be preferentially transported anterogradely, and only on occasion have we seen evidence of retrograde labeling. (6) When introduced iontophoretically there is apparently little uptake and/or transport by fibers of passage, and in this respect the PHA-L technique seems to share one of the principal advantages of autoradiographic techniques. (7) The lectin does not seem to be degraded rapidly within cells or their processes, and is still clearly demonstrable at least two weeks after administration. (8) Since PHA-L can be localized with fluorescent as well as peroxidase immunohistochemical techniques it can be used in conjunction with the immunocytochemical methods (e.g. to identify specific transmitters or their synthesizing enzymes) or with the now commonly used retrogradely transported fluorescent dyes.

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224.7 *THE LABELING OF CORTICOSPINAL NEURONS AFTER INTRASPINAL INSERTION OF HORSERADISH PEROXIDASE VARIES WITH THE AGE OF THE ANIMAL.*

E.R. Feringa, H. Lee Vahlsing*. Neurology Research Laboratory, VA Medical Center, San Diego, CA 92161

We previously reported that the number of corticospinal neurons surviving after spinal cord transection decreases 10 and 25 weeks after axotomy. During our examination of control animals in this series we noted that the labeling of corticospinal neurons is less constant in animals 11 weeks old than in animals which are 16 or 31 weeks old. As a result, we undertook experiments to study the labeling of corticospinal neurons at various time intervals in normal isogeneic rats.

Attempts to label corticospinal neurons by intraspinal placement of horseradish peroxidase in animals less than one week old has resulted in no labeling seen. By 11 weeks postnatal, labeling is highly variable but at least some animals have labeling similar to that seen in older rats. Animals 16 and 31 weeks old had increasingly congruent labeling profiles.

Graphs illustrating the number, pattern, and variability of cells labeled at different ages will be demonstrated.

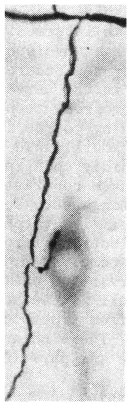
224.8 RETROGRADE AXONAL TRANSPORT OF H^3-NORADRENALINE (NA) *IN DISSOCIATED SYMPATHETIC NEURONS IN VITRO.* M.E. Schwab* and H. Thoenen, (SPON: H. Holländer) Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, 8033 Martinsried, West Germany.

As well as the cycles of exocytosis and endocytosis, synaptic vesicles in nerve endings undergo a turnover which implies the supply of new constituents from the cell body by anterograde axonal transport, and the retrograde transport of 'worn-out' components back to the cell for final break-down. In adrenergic neurons, vesicles are characterized by mechanisms for the uptake (reserpine-sensitive amine pump) and storage of very high amounts of NA (NA-ATP complex, ATP-pump).

When H^3-NA (0.5×10^{-6}M) was added to the axons of dissociated sympathetic neurons from newborn rats grown in a culture dish that separates the neurites from their cell bodies by a Teflon ring sealed with silicon grease (Campenot, PNAS 74:4516, 1977), H^3-NA appeared in the cell bodies following retrograde transport. This transport was fast (min 2-3 mm/h) and blocked by vinblastine. Presynaptic α- or β-receptors were not involved, as neither yohimbine nor propranolol interfered with the transport of NA. Uptake of H^3-NA into the terminals by the plasma membrane amine pump was, however, required as the retrograde transport of NA was blocked by cocaine and desmethylimipramine. Interestingly, reserpine, while completely blocking the accumulation of H^3-NA in the axons, decreased the retrograde transport of H^3-NA only slightly. Electron microscopy of normal and reserpine-treated axons during the retrograde transport of 5-hydroxy-dopamine showed that the amine was present mostly in large vesicles (diam. 700-1000 Å), resembling large dense-core vesicles. As these vesicles have the ability to form a storage complex of H^3-NA or of 5-hydroxy-dopamine that is stable enough for retrograde transport over 3-10 mm, they may represent the population of synaptic vesicles returning to the cell bodies.

224.9 TRANSNEURONAL TRANSPORT OF HRP FOLLOWING INTRA-AXONAL INJECTION OF CAT VESTIBULAR NEURONS. R. Baker & A. Grantyn*. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016 and Carl-Ludwig-Institut f. Physiol., Leipzig, GDR.

Convincing evidence for transneuronal transport of HRP after injection into single cells has recently been obtained by Hongo et al (Biomed. Res. 2: 722-727, 1981) and Triller and Korn (Exp. Brain Res. 43: 233-236, 1981). The present study confirms and extends their hypothesis that a unique exo/endocytosis occurs at synaptic sites. Axons of vestibular neurons were identified and injected with 10% HRP. Successful transneuronal labeling was dependent upon a number of factors: a) anesthesia without barbituates; b) injection sites close to the terminal arborization in the oculomotor nuclei (OcN); c) 10-30 mins of iontophoretic current (total 200-400 nA.mins); and d) sensitive HRP histochemistry. The results are best illustrated by the quantitative analysis of two vestibular neurons that clearly labeled 153 target cells (71 & 82) in the Int. N. Cajal (48), N. fields of Forel (15), OcN (74), and Supra-OcN (16). The frequency of transneuronal labeling was roughly 10% as calculated from the observation that of 3000 synaptic swellings 292 were judged to contact the above 153 neurons (See Fig). Smaller cells (<20μ) were labeled in preference to larger neurons independent of distance from injection site. Transneuronal transport was typically associated with collaterals closest to the main axon. Synaptic swellings were largely



(<90%) on the soma or proximal dendrite. Irrespective of the interface site or size, there was no localized postsynaptic HRP accumulation indicating rapid diffusion. Frequently, two neurons were labeled by boutons from the same terminal collateral yet other nearby bouton clusters were ineffective even though their density (size, number, extent, etc.) appeared much more advantageous for transport. Reconstruction of extensive axonal arborizations distributed widely throughout several target nuclei showed no obvious correlation between transneuronal transport and local collateral patterns thereby ruling out any general reason, be it physiological or pathological, for HRP release. In conclusion, our data is consistent with preferential transport of HRP at synaptic sites via a novel exo/endocytotic process that apparently avoids extracellular space; however, the conditions for selective transneuronal transport by some terminals, and not others, isn't obvious. Supported by NS13742.

224.10 OSMIUM IMPREGNATION OF THE AXOPLASMIC RETICULUM. James D. Lindsey and Mark H. Ellisman, Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla, CA 92093.

Osmium impregnation is a useful technique for studying the three dimensional architecture of the Golgi apparatus cis face and its associated tubule systems. We have found that with minor modifications this technique also proves to be useful for studying the three dimensional structure of the axoplasmic reticulum. Spinal ganglia and spinal nerves from Rana catesbiana were fixed in 2% unbuffered osmium tetroxide for 1 hr. and then impregnated in the same solution at 37°C for 35 hrs. Primary fixation temperatures were varied from 0 to 37°C. The tissue was subsequently dehydrated and embedded in Epon-Araldite. Thin and thick sections were examined with conventional and high voltage electron microscopy. In both ganglia and nerve, the best preservation was achieved with primary fixation carried out at 4°C. However, only the Golgi apparatus cis face and associated tubules in the cell bodies, and no structures in the axon, were impregnated. At higher temperatures (20-30°C), the somal rough endoplasmic reticulum and the axoplasmic reticulum were frequently impregnated. Fixation quality was acceptable, with membranous structures well preserved at these elevated temperatures. At a given temperature, the reticulum was more readily impregnated in unmyelinated axons than in those with moderate or, in particular, with heavy myelination. When the primary fixation temperature was raised to 37°C, some discrete cisterne in unmyelinated axons were occasionally impregnated. In myelinated axons, impregnated discrete cisternae were seen only very rarely. In all axons at all temperatures, impregnated mitochondria and multilamellar bodies were not seen. When examined in 0.5-2 μm thick sections, the reticulum appears as an extensive anastomotic network of tubules with occasional varicosities. Because of the very high contrast between impregnated and nonimpregnated structures, this technique lends itself especially well to thick section analyses of the reticulum.

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224.11 NEUROTUBULE-INITIATING COMPLEX AND FORMATION OF A DENDRITIC CYTOSKELETON. D.E. Hillman and S. Chen. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016

The origin of microtubules emerging from neuronal somata to form the core of dendritic processes has now focused on the centriole as the result of immunofluorescent techniques. Purkinje cells of the cerebellum have as many as 800 tubules emerging from the soma into a single dendritic tree. Morphological correlates for the source of the dendritic cytoskeleton were analyzed in conjunction with the initiating center for microtubules. Ultrathin serial sections were used to obtain sequential electron micrographs throughout the apical soma and base of the main dendrite in developing Purkinje cells. A highly regular arrangement of organelles were found in the apical zone of the soma. The center of this complex had a pair of centrioles and the periphery was bounded by an array of Golgi bodies positioned end-to-end with the secretory surface facing inward to form a continuous sphere-like shell. Each centriole of the pair had different characteristics. One consisted of a tubular array capped by endoplasmic reticulum over the entire distal pole. The other centriole lacked a covering and had a short tubular array. Each basal body of the pair had a pericentriolar complex that consisted of electron-dense, granular masses that are positioned irregularly over the length of its surface. These masses of the pericentriolar body each had at least three to ten microtubules streaming away from the body in various directions. After leaving the Golgi cisternae the tubules joined others to form regular bundles to enter the dendrite. Within the ring formed by the Golgi complex were a number of densely stained bodies having a texture with staining similar to the pericentriolar body. These bodies were scattered as well as aggregated into clusters within the ring. In fortunate sections, it was noted that single tubules end in the dense mass. On occasion, pairs of these structures each had one associated tubule. This analysis shows that the initiating center for dendritic microtubules is a proliferative complex of pericentriolar bodies. As tubules form on the base of centrioles, they become dislodged by disaggregation of portions of the pericentriolar body. Thus a remnant of the pericentriolar body was carried into the cytoplasm. The organization of the tubules into patterns for dendritic processes did not appear to be a function of this complex; however, the apical positioning of the Golgi complex was consistently found at the base of the main dendrite. Supported by USPH grants NS13742 & HD10934.

224.12 GLIAL PROTEINS TRANSFERRED INTO THE SQUID AXON CAN BE SEPARATED INTO DISTINCT GROUPS BY DENSITY GRADIENT FRACTIONATION. M. Tytell. Marine Biological Lab., Woods Hole, MA 02543 and Dept. of Anat., Bowman Gray School of Med., Wake Forest Univ., Winston-Salem, NC 27103.

A subset of proteins synthesized in the adaxonal glia are transferred into the squid giant axon. These transferred glial proteins (TGPs) include actin and previously were shown to behave as a sedimentable complex that is insensitive to hypotonic shock or treatment with 1% Triton. To examine further the sedimentation properties of the TGPs, they were analyzed by centrifugation through a discontinuous sucrose gradient followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the gradient fractions. The TGPs were labeled by incubating 3 cm of the giant axon and its glial sheath in ^{3}H-leucine (1mCi/ml) for 3-6 hrs. The axoplasm containing the labeled TGPs was extruded and homogenized in an isotonic buffered solution. The homogenate was layered on top of a discontinuous sucrose gradient (0.5, 1.0 & 2.5 M or 0.05, 0.5 & 2.5 M) and centrifuged at 190,000 x g_{max} for 4 hrs at 20° C. The gradient was divided into 6 fractions plus the pellet and each was processed for SDS-PAGE followed by fluorography.

The labeled TGPs in axoplasm homogenized in KF or NaF buffer, which preserves native protein associations, were distributed among 3 groups with different sedimentation properties. Group 1 (45-55% of the total labeled TGPs) mainly sedimented to the interface between the homogenate and the first sucrose layer. Group 2 (15-20% of total) sedimented to and partially past the interface between the gradient layers 1 and 2. Group 3 (25-35% of total) sedimented through the 2.5 M sucrose layer and most pelleted to the bottom of the tube. SDS-PAGE revealed that each group had distinctive polypeptides, although some were present in more than one group. For example, actin was present mainly in groups 1 and 3, whereas a 68,000 dalton polypeptide that we have called traversin was found mainly in group 3.

Labeled TGPs in axoplasm homogenized in KSCN buffer, which disrupts native protein associations, were broadly distributed in the top 2 layers of the gradient, but about 10% of the radioactivity corresponding to the TGPs still pelleted. Two pairs of polypeptides (200,000 and 68,000 daltons) were enriched in the pellet. These KSCN-resistant polypeptides may be partly responsible for the sedimentation characteristics of the other TGPs.

These results suggest that the TGPs in axoplasm may exist as 3 distinct macromolecular complexes. Density gradient centrifugation may allow the TGP complexes to be separated from other polymers of axoplasm and examined morphologically to gain insight into their function. (Supported by an MBL Steps Toward Independence Fellowship.)

224.13 GLIAL-NEURONAL INTERACTION IN THE TRANSPORT OF ^{3}H-PROLINE-LABELED MOLECULES IN CAT MEDULLA. N. Contos and K. J. Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

It is well known that the dorsal column nuclei (DCN) send projections to the inferior olive (IO) along the internal arcuate fiber pathway. When ^{3}H-leucine (leu) or ^{3}H-proline (pro) is injected into DCN and the tissue processed for light microscopic autoradiography, both tracers produce labeling over IO (Berkley, 1975).

Electron microscopic autoradiography has shown that whereas both neurons and macroglial cells are densely labeled by ^{3}H-leu at the injection site in DCN, only macroglial cells are densely labeled by ^{3}H-pro (Molinari and Berkley, 1981). In addition, these patterns persist for several millimeters along the internal arcuate pathway (Contos et al., 1981). These results suggest that molecules labeled by ^{3}H-pro follow a glial track from DCN to IO whereas those labeled by ^{3}H-leu follow a neuronal track.

In one test of this hypothesis, samples of tissue were taken at several levels along the length of the internal arcuate pathway from DCN through IO following (24 hrs) injections of ^{3}H-leu or ^{3}H-pro into DCN. The samples were prepared for electron microscopic autoradiography, and the grain distribution analyzed qualitatively and in some cases quantitatively using a simple grain density analysis.

For ^{3}H-pro-labeled samples, the distribution of grains shifted along the length of the pathway. In DCN and adjacent parts of the internal arcuate pathway, grains were located mainly over macroglial elements (e.g. myelin, oligodendrocyte soma, astrocyte soma). Relatively few grains were located over axoplasm. Further along the pathway, glial elements remained labeled, but axoplasm became more densely labeled. Within IO, synaptic terminals were the most densely labeled profiles, but glial elements such as myelin were also labeled. The grain distributions in comparable ^{3}H-leu samples were different from those in the ^{3}H-pro samples. In ^{3}H-leu samples, neuronal elements (such as axoplasm) were more densely labeled and glial elements were much less densely labeled.

These findings are consistent with other data demonstrating that the transport of ^{3}H-pro-labeled molecules is less sensitive to colchicine than the transport of ^{3}H-leu-labeled molecules (Berkley and Contos, 1982), and that no transport occurs when ^{3}H-pro is injected directly into fiber tracts (Berkley et al., 1981). Taken together, the results suggest that ^{3}H-pro-labeled molecules are transported from DCN to IO by a mechanism that involves a close interaction between certain glial cells and internal arcuate axons. In contrast, ^{3}H-leu-labeled molecules are transported along a predominantly axoplasmic track.

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224.14 STRUCTURAL AND SPECTROFLUOROMETRIC ANALYSIS OF PORPHYRIN-CONTAINING GLIAL CELLS IN MOUSE OPTIC NERVE. L.I. Terr and L.P. Weiner. Dept. of Neurology, Univ. Southern Calif. Sch. of Med., Los Angeles, CA 90033.

Porphyrin-containing astrocytes in the periventricular zone of the brain and astrocyte-like cells in the intermediate lobe of the pituitary gland have been identified in previous microscopic and microspectrofluorometric studies.The present report describes the presence of similar cells in the optic nerve.Fluorescence spectroscopy has been employed to investigate their orange-red fluorescence found in cryostat sections mounted in glycerol. The measurements were performed with an automated NanoSpec/10 Spectrofluorometer (Nanometrics Inc., Sunnyvale, Calif.), which utilizes a gallium arsenide photomultiplier. The emission spectrum between 550 nm and 800 nm was recorded. A number of porphyrins, namely, uroporphyrin, coproporphyrin, and protoporphyrin-IX, were used as standards. Comparison of the spectra obtained showed that the fluorescence in the optic nerve is possibly due to the presence of porphyrins. Although the wave lengths of all peaks were the same in these experiments, the relative intensity of the fluorescence of individual sources varied over a rather wide range. This result may reflect the accumulation of different amounts of porphyrins in each fluorescing structure as well as variations in the relative content of different porphyrins within the same structure. A positive reaction for endogenous peroxidase was observed in the optic nerve. The appearance, localization, and pattern of distribution of the reaction product was similar to the results seen with the fluorescence microscope. Since porphyrin is an intermediate product necessary for synthesis of such peroxidases as myeloperoxidase, it may be suggested that both findings reflect the occurence of the biosynthesis of porphyrin-containing enzymes in the nerve. Phase contrast, dark field, light, and electron microscopy techniques demonstrated that the fluorescence is emitted by coarse inclusions in the cytoplasm of astrocytes. Indeed, under dark field illumination, the inclusions can be identified by rapidly changing objectives from dark field to fluorescence. The phase contrast microscopy showed that cells with inclusions have long processes. Staining the semithin sections revealed the astroglial nature of the cells containing the inclusions. Under the electron microscope, the inclusions appeared as dense bodies of extemely variable shape. The absence of outer membrane(s) in most observations was a marked feature of the inclusions. This feature as well as the accumulation of porphyrin distinguishes these inclusions from lipofuscin which has many similar morphologic characteristics. These unique astrocytes and cells from the periventricular zone and pars intermedia may have a common origin.

Supported by the Janice Gram Memorial Fellowship.

224.15 AXOPLASMIC INCORPORATION OF AMINOACIDS EXCEEDS THAT OF THE PERIKARYON. Jaime Alvarez* (SPON. N.C. Inestrosa) Lab. Neurocitología. Univ. Católica. Santiago, Chile.

The neurone is believed to be nourished by the perikaryon. At first sight, the enormous size of the axoplasm seems too much a load for the tiny cell body. I studied therefore the incorporation of aminoacids in the goldfish Mauthner axon to determine (1) its magnitude, (2) the life span of the resulting material and (3) whether this material is transported. Aminoacids were locally injected into medulla and spinal cord and followed with radioautography at different survival times.

At the site of injection, Mauthner axoplasms were weakly and neighbouring perikarya strongly labelled (ratio 1:71). The Mauthner perikarya, -away from the site of injection- were practically free of label. Cycloheximide depressed the local radioautographic response of the axoplasm. Artefactual retention of aminoacids by the axoplasm was ruled out by in vivo chase with unlabelled aminoacids and by replacing the formaldehyde of the histological routine with TCA. High resolution radioautography showed the label associated with the axoplasmic sap rather than mitochondria or endoplasmic reticulum. The time course of the axoplasmic incorporation did not have a lag time, making unlikely a glial origin of the axoplasmic label. The profile of the response on the axoplasm showed a peak at the site of injection and a steep decrease in both directions. This profile remained unchanged for 5.6 days, but its intensity decreased to 14% of the initial value in the same period.

My findings indicate that the axoplasm incorporate aminoacids weakly, but owing to its enormous volume -1250 times greater than the soma- the total axoplasmic incorporation greatly exceeds that of the perikaryon. The synthesized material, seemingly proteins, does not move and has a life span of a few days.

224.16 ENDOGENOUS LABELING OF AXONAL POLYPEPTIDES IN GOLDFISH RETINAL EXPLANTS AND SPINAL ROOTS OF THE RAT. E. Koenig. Div. of Neurobiology, SUNY, Buffalo, NY 14214.

Recent studies by Nixon (Brain Res. 200:69, 1980) indicate that slowly transported proteins in the axon undergo degradation in transit at measurable rates. Nixon observed indirect evidence of local reutilization of labeled amino acids released by catabolism in the absence of an inhibitor of protein synthesis. Local reutilization could account for the perseverance of labeled proteins in the wake of a radioactive peak passing through a region (Black and Lasek, J. Cell Biol. 86:616, 1980). The apparent paradox of relatively short half-lives and long transit times of slowly transported proteins is not readily reconciled without seriously considering the potential contribution of locally synthesized axonal proteins. I have been studying cycloheximide-sensitive labeling of axonal proteins in decentralized axonal fields of goldfish retinal explants in culture, and, in addition, cycloheximide-sensitive labeling of axonal proteins in axons of spinal roots of the rat. In both cases, microscopic samples of axons, free of contamination by ensheathing cells, were analyzed in a gel microslab system developed in this laboratory to separate polypeptides by SDS electrophoresis in the lower ngm range. Proteins were labeled with ^{35}S-methionine in vitro in the presence or absence of 1 mM cycloheximide. Gel microslabs were stained with silver, dried onto glass slides and exposed to x-ray film. Goldfish retinal explant axons, which are free of glial cells in culture, exhibit cycloheximide-sensitive labeling of 5 to 6 components, including α and β tubulins, actin, and a putative 145K neurofilament (NF) polypeptide (a 200K NF polypeptide is not present in goldfish). The β-tubulin is labeled in a disproportionately greater amount than is α-tubulin. Axons from dorsal and ventral roots of the rat show at least 10-12 labeled components during a 4-hour in vitro incubation period. Major polypeptides labeled are α and β tubulins and actin. In addition the 200K and 160K NF polypeptides are also labeled; however, while the 70K NF polypeptide does not appear to be labeled significantly, a slightly faster moving 67K component is labeled. The labeling-pattern of axonal proteins from rat spinal roots is distinctly different from that of myelin sheath proteins, indicating that the sheath was not a significant source of labeled axonal proteins. These results indicate that certain cytoskeletal proteins supplied to the axon by slow transport appear to be supplemented by local synthesis.

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224.17 SITS: A COVALENTLY BOUND FLUORESCENT RETROGRADE TRACER THAT DOES NOT APPEAR TO BE TAKEN UP BY FIBERS OF PASSAGE. L.C. Schmued* and L.W. Swanson (SPON: U. Bellugi). The Salk Institute, La Jolla, CA 92037.

In the past several years Kuypers and his colleagues have introduced a number of fluorescent compounds that can be used much like HRP to label retrogradely the cells of origin of pathways in the central and peripheral nervous systems. These tracers have been found useful because they are sensitive, require minimal tissue processing, and two or more fluorescent tracers may be combined to demonstrate collateral projections. However, several inherent limitations are associated with the use of these tracers. One limitation is that, like HRP, the tracers are taken up by fibers-of-passage as well as by axon terminals. In addition, these tracers tend to diffuse out of retrogradely labeled neurons after either prolonged survival times or after subsequent histochemical processing.

In this abstract we report on a new fluorescent retrograde tracer that does not appear to be subject to the aforementioned limitations. The fluorescent anion SITS (4-acetamido, 4'-isothsiocyanostilbene - 2, 2' disulfonic acid) was used as a 10% solution in distilled water. This solution was either pressure injected (50-200 nl) or iontophoretically applied (negative current) into various regions of the rat brain. After a suitable survival period (2-30 days) the animals were perfused with neutral buffered formalin and postfixed in fixative with 15% sucrose. Thirty µm thick sections were cut, mounted, and covered with buffered glycerol or DPX. Wide-band UV excitation was used for examination.

When injected into a wide variety of terminal fields, conspicuous retrogradely labeled neurons were observed in all appropriate sites. Many discrete light blue fluorescing vesicle-like structures could be found within the cytoplasm of labeled neurons. However, when the corresponding fiber tracts were injected, rather than the terminal fields, retrogradely-labeled cells were not observed. Also, animals allowed a long survival time (30 days) after SITS injections into terminal fields showed brilliant retrograde labeling of corresponding neurons with no diffusion to adjacent neurons or glial cells. In addition, SITS labeling did not appear to be altered appreciably when sections were processed according to standard immunohistochemical, autoradiographic, and HRP histochemical procedures.

In conclusion, it appears that SITS is a sensitive retrograde neuronal tracer that is not taken up by fibers-of-passage, and does not diffuse from labeled cells. Both of these useful features may be due to the fact that the tracer forms covalent bonds with neuronal plasma membranes under aqueous physiological conditions.

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224.18 IS RETROGRADE AXONAL TRANSPORT OF FLUORESCENT DYES DEPENDENT ON IMPULSE ACTIVITY IN NEURONS? W. R. Woodward and B. M. Coull*. Depts. of Neurol. and Biochem., Oregon Health Sciences University, Portland, OR 97201.

Following kainate (KA) lesions in rat dorsal lateral geniculate nucleus (dLGN) which completely destroyed geniculocortical neurons and spared corticofugal fibers (Woodward & Coull, 1982) we observed that corticogeniculate (CG) neurons no longer transported fluorescent dyes such as Nuclear Yellow (NY) retrograde but did transport substances such as D-aspartate and wheat germ agglutinin. We have considered two explanations for the loss of NY transport: (1) KA may partially damage CG terminals resulting in selective loss of dye transport capability. (2) Since CG fibers are likely to be less active by virtue of having lost geniculate input to cortex, NY transport may depend upon impulse activity and use the "non-specific" transport mechanism proposed by Thoenen and Schwab (1979). It is possible to distinguish between these alternatives because (1) suggests that retinogeniculate terminals might be similarly damaged and, therefore, not transport dyes, and (2) suggests that corticotectal fibers are also likely to have reduced impulse activity and, thus, not transport dyes even though their terminals have not been exposed to KA.

We have tested these alternatives in double dye injection experiments. Bilateral injections of Fast Blue (FB, 1% in 2% DMSO) and NY (2% in 2% DMSO) were made into dLGN and superior colliculus respectively following unilateral KA lesions in dLGN. Tissues from striate cortex and retina were examined for evidence of retrograde axonal transport in corticofugal and retinofugal fibers.

We found that in retina, numerous ganglion cells are labeled by dye from dLGN and tectum, and no appreciable differences between labeling patterns on control and lesion sides of the brain are observed. In contrast, retrograde dye transport to cortex is greatly reduced in corticotectal fibers on lesioned compared to control sides. This reduction in corticotectal cell labeling is probably due to changes in transport function and not to differences in injections since retinotectal cells on both sides are comparably labeled in the same experiment and since corticotectal fibers on both sides do transport wheat germ agglutinin to a similar extent.

These results favor the hypothesis that reduction of retrograde dye transport in corticofugal fibers after KA lesion results from a reduction in impulse activity and not from primary neuronal damage. This hypothesis has been directly tested by activating these pathways with cortical stimulation, and the results of these experiments will be presented.

This work has been supported by funds from the Oregon Medical Research Foundation, the Roy L. Swank Foundation and NEI grant 02456.

224.19 CYTOCHEMISTRY AND AXONAL TRANSPORT OF NEURONAL GLUCOSE-6-PHOSPHATASE (G6PASE). R. Broadwell and A. Cataldo*. Dept. of Pathology (Neuropathology), Univ. Maryland Med. Sch., Balt., MD 21201.

G6Pase, the activity of which is a marker for the endoplasmic reticulum (ER) in many cell types, functions predominantly as a phosphohydrolase for converting glucose-6-phosphate to glucose. We reported earlier that in neurons the cytochemical localization of G6Pase activity is demonstrable in the ER of perikarya and dendrites but noticeably less so in the axonal ER under normal conditions (Histochem. Soc. Abstr., 1982). The purpose of the present investigation was to stimulate the axonal transport of G6Pase as detected ultrastructurally by cytochemical localization of G6Pase activity. Sections of the cortex, XIIth and supraoptic nuclei, neurohypophysis, VIIth nerve, and anterior commissure from control mice and mice hyperosmotically stressed by drinking 2% salt water were incubated for G6Pase activity. Glucose-6-phosphate, inorganic pyrophosphate, thiamine pyrophosphate, and β-glycerophosphate served as substrates. Only tissue incubated in the presence of glucose-6-phosphate exhibited G6Pase reactive ER in neurons and glia. In control animals G6Pase activity was particularly sparse in the axonal ER. Reactive segments of the axonal ER were 20-30 nm wide, short in length, and straight or branched. G6Pase activity was not observed in axon terminals. Hyperosmotic stress, which increases the metabolic, physiologic and secretory activities of the hypothalamo-neurohypophysial system (Broadwell and Oliver, J. Cell Biol. 90:474, 1981), resulted in numerous, long profiles of G6Pase reactive ER within neurohypophysial axons and terminals but not in any of the other neuronal systems sampled. No differences were noted in the localization of G6Pase activity within neurosecretory perikarya of supraoptic nuclei from hyperosmotically stressed mice compared to control mice. Many of the neurohypophysial axon terminals containing G6Pase reactive ER exhibited an abundance of autophagic vacuoles. These vacuoles contained cytochemically detectable acid phosphatase (AcPase) activity as did blunt-ended, 50-130 nm wide tubules possessing a smooth or varicose contour within neurohypophysial axons. Morphologically similar tubules were seen to transport horseradish peroxidase (HRP) in anterograde and retrograde directions. Such tubules do not appear to be part of the ER. Our speculation is that the axonal ER contributes membrane for the formation of autophagic vacuoles. The AcPase reactive tubules, derived perhaps from perikaryal secondary lysosomes (Broadwell et al., J. Comp. Neurol., 190:519, 1980), may contribute acid hydrolases to the same autophagic vacuoles. These findings suggest: (1) G6Pase activity is a reliable marker for the neuronal ER; (2) the axonal transport of G6Pase and that of acid hydrolases/HRP occur in different compartments; and (3) a relationship may exist between neuronal G6Pase activity and energy metabolism.

224.20 AN ELECTROPHORETIC ANALYSIS OF SLOW AXOPLASMIC TRANSPORT IN THE RUBROSPINAL TRACT OF THE CAT. J.L. Cova* (SPON: A. Iannone). Depts. of Anatomy and Neuroscience, Medical College of Ohio, Toledo, Ohio 43699.

As a prelude to a study of the effects of axotomy on the axonal transport of proteins in the rubrospinal tract of the cat, the distribution of ^{35}S methionine labelled polypeptides in the intact rubrospinal tract was examined using the techniques of sodium dodecyl sulfate polyacyrlamide gel electrophoresis (SDS-PAGE), fluororadiography and liquid scintillation counting. ^{35}S methionine (1100 Ci/mmol, Amersham) was stereotactically injected (0.8ul) bilaterally (47.0-50.0uCi/red nucleus) into the red nuclei of intact animals. After a twenty day survival period animals were anesthetized with pentobarbital sodium and sacrificed by intra-aortic perfusion with Tyrode's solution (pH7.2). Serial segments (5mm in thickness) of cervical spinal cord containing the dorsal half of the lateral funiculus were homogenized in buffer (10mM Tris, pH 8.0, 5mM EDTA, 1mM o-phenanthroline, 1mM phenylmethylsulfonylfluoride). Trichloroacetic acid precipitates of the homogenates were washed 3 times with ether and resuspended in denaturing buffer (2% sodium dodecyl sulfate (SDS), 0.5% mercaptoethanol, 10mM Tris) and analyzed on 12.5% or 15.0% SDS polyacyrlamide slab gels or gradient SDS polyacyrlamide slab gels (5.0%-12.5%). Dried gels were fluorographed on Kodak XAR-5 film. Exposure was for 14 days at -70°C.

Preliminary evaluation of the distribution of total radioactivity contained in the homogenates of serial segments of spinal cord suggests that ^{35}S methionine labelled polypeptides in the slow phase of axonal transport are associated with 2 waves. Analysis of fluorographs obtained from the homogenates indicates these peaks differ from one another in their polypeptide composition and may represent functionally different components of slow axonal transport.

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224.21 AXOPLASM VOLUME AND MYELIN THICKNESS ARE REDUCED BY COLCHICINE. S. E. Hughes*, H. E. Sloan, L. B. Jones* and B. Oakley. Div. Biol. Sci., Neuroscience Lab. Bldg., Univ. of Mich., Ann Arbor, MI 48109.

The combined lingual-chorda tympani nerve of the Mongolian gerbil (Meriones unguiculatus) was treated with a Silastic nerve cuff containing either colchicine (1% w/v) or no colchicine. The 1% colchicine concentration was chosen because it was adequate to produce the characteristic disorientation and increased prominence of neurofilaments, loss of microtubules and impairment of axonal transport of cholinesterase. Three days of exposure caused physiological impairment of taste responses in the chorda tympani without eliminating impulse conduction. Quantitative computerized measurements of axon profiles distal to 3 day colchicine cuffs revealed a 23% reduction in myelin period and a 19% shrinkage of axoplasm volume when compared to nerves cuffed without colchicine. The mean major period distance of the myelin sheath was reduced from 14.7 to 11.3 nm. All myelin displayed a constant percentage reduction independent of the number of lamellae, which ranged from 16 to 61. The amount of axoplasm shrinkage in a given axon was unrelated to its fiber diameter. Adjacent axons of similar diameter often displayed different amounts of axoplasm shrinkage. We suggest that the blockage of axonal transport by colchicine is responsible for axoplasm shrinkage, whereas the reduced myelin period may reflect either direct damage to the Schwann cells of the blockage of axonally transported substances required for myelin maintenance.

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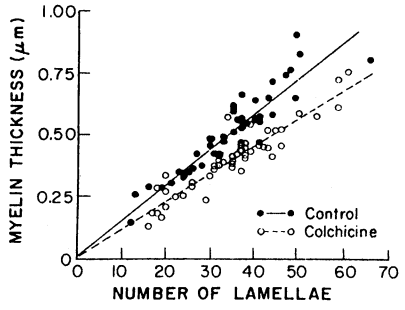

224.22 COMPARISON OF BIOLOGICAL STRUCTURE IN PLATELETS AND NERVE MICROSACS AS SEEN BY X-RAYS AND ELECTRONS. R. Feder,* D. Sayre,* J.L. Costa*, and V. Mayne-Banton* (SPON: C.R. Creveling). IBM T.J. Watson Research Center, Yorktown Heights, NY 10598 and NIMH, Bethesda, MD 20205.

X-ray contact microscopy is a novel technique for the examination of biological specimens. Replicas are made by placing cells in contact with polymethylmethacrylate polymer and exposing the combination to long-wavelength x-rays (carbon K_α radiation at 44Å). The polymer undergoes differential dissolution when developed by immersion in methyl isobutyl ketone, and thus provides a replica of the variations in x-ray density (absorbance) of the specimen. The developed replica is examined by transmission electron microscopy (TEM) to permit high-resolution study of the details of the x-ray image.

To explore the utility of x-ray contact microscopy for the study of amine-storing tissues, we made replicas of air-dried whole mounts of human platelets and nerve-ending particles isolated from the guinea pig cerebral cortex (Creveling, C.R., et al., J. Neurochem. 35:922, 1980). Both cell types contained what appeared to be cytoskeletal elements distributed throughout the cytoplasm. Pairs of x-ray stereo replicas of platelets showed that the skeletal structure was a 3-dimensional array, i.e., an upper and a lower skeletal system with a number of interconnections. In platelets, this structure did not appear to be composed exclusively of actin filaments.

Each replicated specimen was also examined directly in the TEM. Comparison between the TEM appearance and the replicated image showed that electron dense areas and x-ray dense areas did not correspond in subcellular location. Thus conventional TEM and x-ray contact microscopy appear to highlight different types of subcellular structures in platelets and nerve endings.

225.1 RAPID RELEASE OF SUBSTANCE P. E. Floor. Dept. of Physiology, Univ. Massachusetts Medical School, Worcester, MA 01605.

The rate of substance P release from rat brainstem synaptosomes at 37° after depolarization in 100mM K^+ was measured at a time resolution of one second. Synaptosomes were purified by differential centrifugation and banding on a linear 2.5-12% Ficoll/sucrose gradient as described (Floor et al., Neuroscience, in press), suspended in 8 ml KB (135mM NaCl, 3mM KCl, 5mM $CaCl_2$, 2.3mM $MgSO_4$, 10mM dextrose, 20mM HEPES pH 7.3, ~0.2mM (17U/ml) bacitracin) and bubbled with O_2 for 8 min at 37°. The suspension was then loaded into a 10ml syringe attached to a larger syringe containing 0.8ml of 50mM K_2SO_4, 50mM Na_2SO_4, or KB and mounted on a Gilson P-1000 Repetman preloaded with KB+50mM K_2SO_4 or Na_2SO_4 or with KB and warmed to 37°. At zero seconds the synaptosomes were injected into the large syringe and thereby mixed with its contents within ~1/4 second. At 1,2,3,4, and 5 seconds as determined with a metronome 1ml samples were dispensed and filtered (~0.1 sec) through Whatman GF/F glass fiber filters. The filtrates were heated at 90° for 5 min and assayed for SP.

Levels of free SP at 1-5 seconds were as follows (mean ± SE for the five time points): 50mM K_2SO_4, 142 ± 2 fmol/ml; 50mM Na_2SO_4, 87 ± 2; Ca^{++}-free KB + 1mM EGTA, 61 ± 1; KB, 70 ± 3. These levels were reproducible; from 5 experiments the standard error of the mean SP level at each time point was about 5% of the mean. The recovery of a 10-fold excess of synthetic SP added to unheated filtrates taken at 5 seconds after K^+ or Na^+ treatment and incubated 10 min at 37° was >98%. The amount of K^+-stimulated SP release, 55 fmol/ml, was 2.8 ± 0.2% (SE, n=5) of the total synaptosomal SP. About the same amount of SP, 75 + 20 fmol/ml or 3.5 ± 1% (SE, n=2) was released into the supernates from parallel batches of synaptosomes bubbled continuously with O_2, depolarized for 1 min at 37° with 50mM K_2SO_4, and centrifuged. The amount of SP release was also similar, 3.1 + 0.1% (SE, n=6), from synaptosomes placed on a filter, superfused with 37° KB at 6ml/min and depolarized with 50mM K_2SO_4.

These experiments demonstrate that all synaptosomal SP release induced by a single K^+ depolarization occurs in less than one second. Release probably did not stop because of inactivation of calcium entry (Blaustein, J. Physiol., 247, 617), O_2 depletion (Booth & Clark, Biochem. J., 176, 365), synaptosomal volume changes, or release of inhibitory substances (superfusion experiments). Release may be inhibited by excess calcium; or activation of additional SP for release may require repolarization of the terminals.

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225.2 EXCITABILITY CHANGES IN STRIATAL DOPAMINE TERMINALS INDUCED BY AUTORECEPTOR STIMULATION AND BLOCKADE. J.M. Tepper*, S.J. Young*, and P.M. Groves. Dept. Psychiatry, Univ. Calif. San Diego, La Jolla, CA 92093.

Evidence suggests that dopaminergic neurons possess presynaptic receptors for their own neurotransmitter located at or near their axon terminals, which are believed to participate in the self-regulation of transmitter biosynthesis and release. Such regulation may be achieved, in part, by changes in the electrical properties of the terminal. We have examined the effects of the local infusion of presumed dopamine autoreceptor ligands and alterations in impulse flow on the excitability of dopaminergic axon terminals. The excitability of axon terminals of dopaminergic nigral neurons to electrical stimulation was measured by determining the threshold, or stimulus current just sufficient to evoke antidromic responses on 100% of stimuli delivered from the ipsilateral neostriatum in urethane anesthetized, immobilized and artificially respired rats. Local microinfusions of apomorphine, amphetamine, haloperidol, fluphenazine, potassium or vehicle were made into the site of stimulation (0.0625/ul/min for 5 min) and the threshold was redetermined. Both amphetamine and apomorphine (10^{-6}M to 10^{-5}M) consistently increased the threshold. The effects of the dopamine antagonists were more complex. Fluphenazine (10^{-5}M) generally produced decreases in threshold, but the effects of haloperidol (10^{-7}M - 10^{-5}M) were equivocal. Infusions of KCl (50mM - 100mM) led to consistent decreases in threshold. Infusions of vehicle did not change threshold. Infusions of apomorphine (10^{-6}M) into the median forebrain bundle did not change the threshold from this stimulating site. Thresholds also varied as a function of the frequency of impulses. Thus, stimulation of dopaminergic axons in the median forebrain bundle (1-10/sec) increased threshold, an effect which could be blocked by a local infusion of haloperidol (10^{-7}M) into the neostriatal stimulating site. Changes in threshold also correlated with changes in spontaneous firing rate. Increases in threshold following agonist infusion and increased impulse flow are believed due to increased stimulation of terminal autoreceptors, while decreases following antagonist infusion are due to autoreceptor blockade. The equivocal results with haloperidol may be due to a local anesthetic effect offsetting the increased excitability due to receptor blockade. These results are consistent with the hypothesis that dopaminergic neurons possess terminal autoreceptors which are active under physiological conditions in situ, and suggest that the mechanism of autoinhibition of transmitter release may be related to increased polarization and/or conductance of the axon terminal which is reflected by decreases in terminal membrane excitability. (Supported in part by Grant DA 02854 and Research Scientist Development Award DA 00079 from N.I.D.A. to PMG).

225.3 MULTIPLE ACTIONS OF SEROTONIN UPON FROG PRIMARY SENSORY AFFERENT CELL BODIES AND TERMINALS. G.G. Holz IV and E.G. Anderson. Dept. Pharmacol., Univ. Ill. Coll. of Med., Chicago, IL 60612.

Bulbospinal serotonergic projections terminating within the spinal cord dorsal horn exert an inhibitory influence upon some classes of interneurons. This action may reflect direct pre- and/or post-synaptic actions of serotonin (5-HT) upon primary sensory afferents and spinal interneurons respectively. To assess a possible presynaptic action of 5-HT upon primary sensory afferents, sucrose gap recordings were obtained from superfused frog dorsal root ganglia (DRG) and tetrodotoxin (TTX, 1 μM) treated hemisected spinal cords (HSC) in vitro.

5-HT (50 μM - 1 mM) produced a slow dose dependent depolarization of primary afferent terminals (0.1 - 1.0 mV; ED_{50} 140 μM) and cell bodies (0.1 - 0.4 mV) as recorded from the lumbar dorsal roots of attached hemisected spinal cords or dorsal root ganglia. A pronounced tachyphylaxis to repeated 5-HT administration necessitated allowing a 60 minute recovery period between each dose. No effect upon desheathed dorsal root fibers was observed. In the TTX treated HSC preparation the magnitude of the maximal 5-HT depolarization was consistently about 65% of that seen in unpoisoned preparations. This TTX resistant response represents a direct action of 5-HT upon primary afferent terminals since it remained upon addition of 2 mM Mn^{++} to the Ringers solution. Microinjection of a small bolus of concentrated 5-HT into the superfusion stream revealed an additional fast depolarizing component preceding the slower component of the HSC response. In unpoisoned preparations, low concentrations of 5-HT (0.1 - 50 μM) altered primary afferent excitability as manifested by an enhancement of spontaneous antidromic dorsal root discharge and evoked dorsal root reflexes.

The sensitivity of primary afferent cell bodies to 5-HT lends further support for a direct action upon these neurons. The DRG response exhibited a pronounced tachyphylaxis but differed from the afferent terminal response in that it was irreversibly blocked in 4 of 6 cases by Mn^{++}, while in 2 of 6 cases there was a reversible attenuation. In 4 of 14 cases the response to 5-HT was biphasic with a late hyperpolarization.

These findings demonstrate a direct influence of 5-HT upon frog primary afferent terminals and cell bodies. The actions of 5-HT upon sensory afferents suggest a role for 5-HT as a modulator of primary afferent neurotransmission or neurosecretion. Whether the actions are the basis for presynaptic inhibition or facilitation remains to be elucidated. However the multicomponent nature of the responses (fast and slow, depolarizing and hyperpolarizing) suggests a complex influence on primary afferent transmission. (Supported, in part, by NIH-NS 14985)

225.4 PRIMARY AFFERENT DEPOLARIZATION AND PRESYNAPTIC INHIBITION OF Ia EPSPs DURING POST-TETANIC POTENTIATION OF GROUP Ia AFFERENTS. A. Lev-Tov*, J. W. Fleshman and R.E. Burke. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205

Conditioning volleys (4 volleys, 200 Hz, 2xT) in group I afferents in the posterior biceps-semitendinosus (PBST) nerve depolarize medial gastrocnemius (MG) group Ia primary afferents (PAD) and depress homonymous MG Ia EPSPs. We examined these phenomena in pentobarbital anesthetized cats before and after tetanization (500 Hz, 20 s, 2xT) of the MG group I afferents by recording intracellularly from MG motoneurons or Ia afferents, or by testing Ia afferent excitability to direct stimulation. Three sets of results were obtained. First, PBST-conditioned and unconditioned MG Ia EPSPs were elicited in alternating trials (inter-trial interval 1 s) before and after MG tetanization. Relative EPSP depression was calculated as the ratio of conditioned to unconditioned responses. This ratio increased by a factor of 2 immediately after cessation of the PTP-producing tetani and decayed back to pretetanic values in 1-2 min. The post-tetanic increase in Ia EPSP depression was not accompanied by significant shortening of the EPSP decay phase, which mitigates against a post-synaptic mechanism. Second, intraspinal (lamina IX) stimulation of MG Ia afferents, with PBST conditioning stimulation on alternate trials, revealed a 3-fold post-tetanic increase in the ratio of PBST conditioned to unconditioned Ia antidromic responses recorded from the cut MG muscle nerve. The afferent hypoexcitability during PTP was virtually cancelled by PBST conditioning and this relative increase in Ia excitability with PBST conditioning decayed in parallel with the hypoexcitability of the unconditioned responses. Third, PAD elicited by 4 PBST volleys (200 Hz, 2xT, repeated at 1 s intervals) was recorded intracellularly from MG Ia afferents in the dorsal column before and after tetanization of the MG nerve. Intra-axonal PAD was enhanced by a factor of 3 following an MG tetanus and then decayed to pretetanic level in 1-2 min. The peak of potentiated intra-axonal PAD was delayed and the decay phase shortened by appearance of a hyperpolarizing overshoot (PAH), which disappeared with time more rapidly than the enhanced PAD. The time courses of the post tetanic increase in effective EPSP depression, enhanced group Ia afferent excitability and intra-axonal Ia PAD amplitude were strikingly similar. This interrelation is best explained by assuming that PAD is generated by neurotransmitter released from axo-axonic synapses. Enhancement of the effective presynaptic inhibition after the tetanus might serve to prevent excessive post-tetanic transmitter release and a subsequent neurotransmitter depletion and/or inactivation of presynaptic release sites.

225.5 LARGE SETS OF INDIVIDUAL EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPS) RECORDED IN SINGLE MOTONEURONS, J. Mathis*, E. Henneman and H.-R. Lüscher, Dept. of Physiology, University of Zürich, CH-8028 Zürich, Switzerland.

Methods. Multiple projections of spindle group Ia and II fibers to single homonymous motoneurons (MNs) were studied in anesthesthized cats by means of spike-triggered averaging. Stretch evoked impulse activity was recorded simultaneously with 5 pairs of electrodes from 5 uncut dorsal root filaments (DRFs), dissected until each contained 2-6 spindle afferents from the medial gastrocnemius (MG) muscle. Stretch of the MG was adjusted to cause continuous discharges in all these units. The trains of signals recorded from these 5 DRFs were stored simultaneously in 5 different channels of a tape recorder and the EPSPs they evoked in a homonymous motoneuron were stored in a 6th channel. During repeated playback the mixture of spike trains from one DRF channel were led to a dual time-voltage window discriminator adjusted to trigger only on one of the spike trains. A signal averager, whose trace was triggered from the window discriminator, received its input from channel 6 containing all the EPSPs recorded from the MG motoneuron. By means of spike-triggered averaging EPSPs elicited by impulses in up to 17 afferent fibers could be averaged out of the recording from a single motoneuron. Furthermore, the functional connectivity of the same set of afferent fibers to 15 different MNs could be studied in the same experiment.

Results.

(1) The amplitudes of single fiber EPSPs recorded within the same MN were directly related to the conduction velocities of the afferent fibers, suggesting that a large afferent fiber gives off more active synapses to a particular MN than a small, slowly conducting fiber.

(2) Rapidly conducted impulses elicited EPSPs in more homonymous MNs than slowly conducted impulses did.

(3) Small MNs (gauged by their axonal conduction velocity and input resistance) tend to receive fewer transmitting projections than large MNs. Thus, the sizes of the afferent fiber and of the MN both play roles in determining connectivity between muscle spindle afferents and MNs.

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225.6 SIMULTANEOUSLY ACTIVE AND INACTIVE SYNAPSES IN THE TERMINAL ARBORIZATIONS OF SINGLE IA FIBERS ON MOTONEURONS, H.-R. Lüscher, E. Henneman and J. Mathis*. Dept. of Physiology, University of Zürich, CH-8028 Zürich, Switzerland.

Abundant evidence indicates that conduction block may occur at branch points in peripheral axons. We have offered indirect evidence (Neurosci. Abst. 6: 601 and 575, 1980) that transmission failure somewhere in the terminal arborizations (TAs) of Ia fibers may be largely responsible for the various states of depression and facilitation observed at Ia-motoneuron (MN) junctions. Observation of simultaneously active and inactive synapses arising from a single Ia fiber might provide interesting clues to the nature of such transmission failure. When many Ia projections are studied simultaneously, as described in another abstract and adjacent poster, most of the EPSPs recorded with the aid of spike-triggered averaging (STA) have one peak and a simple time-course, indicating that the active synapses are all located at similar distances from the soma. A few EPSPs have composite time-courses revealing that some Ia fibers give off terminals to more than one area of the MN, as Burke's HRP studies show. When a set of EPSPs was re-averaged after a 30-60 min interval and the MN was depolarized by 10-15 mV (but was still not below -55 mV), the amplitude, latency and shape of a few EPSPs changed markedly, whereas the remaining, simultaneously recorded EPSPs were unaltered. The electrical parameters of the impaled MN were estimated from the decay time-course of the voltage transient resulting from an intracellular current pulse applied in the soma and a theoretical shape-index curve for all of the individual EPSPs recorded from that MN was calculated. The electrotonic locations of the active synapses were determined from the shape index of each EPSP or its components.

Results.

The changes in amplitude, latency and time-course in re-averaged EPSPs indicate that some Ia fibers had "silent" synapses throughout the period of the first STA, which became active in the second STA. The existence of simultaneously active and inactive synapses in the TA of a single fiber was thus disclosed. It has been proposed that inactive synapses are due to failure of the transmitter release mechanism. Since we are not aware of synapses in the CNS which have transmitter release probabilities of 0 for long periods of time and branch point block definitely occurs in peripheral axons, we favor the hypothesis that silent synapses and their activation are best explained by conduction failure and its relief at sites where the safety factor for propagation of action potentials is low.

Supported by SNF 3.536.079 to H.-R. L.

225.7 FREQUENCY DEPENDENCE OF Ia-MOTONEURON EPSPs IS CORRELATED WITH MOTONEURON SIZE. W.F. Collins III, M.G. Honig and L.M. Mendell. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, N.Y.11794

We have examined the response of single Ia fiber-motoneuron (MN) connections in acute spinal (T13), anesthetized cats to Ia fiber impulse trains of 250 stimuli at various frequencies. Single Ia fibers, penetrated with microelectrodes at the level of the dorsal roots, were stimulated by current injection. We have previously reported (Collins & Mendell, 1981, Neurosci. Abstr. 7: 438) that averages of 250 EPSPs at a given connection diminish in amplitude at high stimulus frequencies due to a decrease in EPSP amplitude during the train. The lowest frequency at which this depression was observed varies among connections from 30Hz to 200 Hz. At some connections, EPSP amplitudes actually increase during trains of intermediate frequencies. We have examined the organization of this frequency dependence in terms of the properties of MNs and Ia-MN connections. Intermediate stimulation frequencies (50-100Hz) were used because this is where connections display maximal differences. Further, since fluctuations in EPSP amplitude necessitate that averaging techniques be used and since EPSP amplitudes change only gradually in this frequency range, the mean amplitude of the first 25 EPSPs provided an indication of EPSP size at the beginning of the train. For a given frequency at each connection, we computed the ratio of the mean amplitude of all 250 EPSPs to the mean amplitude of the first 25 EPSPs. The ratios for the 4-7 trains delivered at frequencies between 50 and 100Hz were averaged and converted to a percent change. We found a wide variation among connections with some showing an increase (facilitation) during the trains as high as 13% and others a decrease (depression) of as much as 20%. The percent change was directly correlated with MN size, as indicated by rheobase. Small (low rheobase) MNs tended to show depression during trains of 50-100Hz whereas large (high rheobase) MNs tended to show facilitation. There was also an inverse correlation with EPSP amplitude; large EPSPs exhibited the most depression. It is not clear whether MN size (or motor unit type) or EPSP amplitude (quantal content) or both determines the observed frequency dependence since EPSP amplitude and MN size are themselves correlated.

These results may indicate inherent differences in the properties of Ia connections on small and large MNs (e.g. receptors, branch point invasion, quantal content, kinetics of transmitter replacement, etc.). Alternatively, or in addition, high levels of transmitter release may result in more transmitter depletion, desensitization, etc. An interesting possibility is that whatever factor(s) results in large Ia-MN EPSPs (motoneuron size, motor unit type, transection, etc.) may predispose that synapse to exhibit depression. (Supported by NIH grants NS06407 to WFC, NS06427 to MGH and NS16996 and NS14899 to LMM)

225.8 STEADY STATE SYNAPTIC DEPOLARIZATION IN SPINAL MOTONEURONS: RELATION TO INPUT RESISTANCE AND MOTOR UNIT TYPE. J.B. Munson, F.R. Morales, G.W. Sypert and J.E. Zengel. Univ. of Fla. Coll. of Med., Gainesville, Fla., 32610.

Many authors have assumed that motoneuron input resistance (R_N) should be an important determinant of the amplitude of postsynaptic potentials (PSPs). Indeed across the spectrum of motor unit types a weak correlation exists between R_N and PSP amplitude. However this relation was not found within motor unit types, and PSP amplitude differences between motor unit types remained after any contribution of R_N was statistically removed by analysis of covariance, demonstrating that R_N is not a primary determinant of PSP amplitude (1, 2).

Other authors have emphasized that input impedance (Z_N; which embraces membrane resistance and capacitance and the spectral components of the exceedingly brief synaptic current transient) is the relevant parameter determining PSP amplitude (3, 4). This could explain the observed independence of PSP amplitude and R_N (1, 2).

Membrane capacitance, which contributes to Z_N, should be a determinant of transmembrane potential (E_m) only when E_m is changing over time. Accordingly we wished to test whether R_N might determine E_m during steady state synaptic depolarization, as it does during prolonged intracellular current injection. E_m of MG motoneurons was recorded during 3 sec of 300 Hz, 50 μm vibration of the combined LGS tendon and muscle. Motor unit properties were then determined (1). The amplitude of the resulting steady state Ia synaptic depolarization of MG motoneurons for each motor unit type increased in the order FF (0.9 mV), FR (2.2 mV), S (3.0 mV; $p < .01$ for FF vs. FR or S). Within the FF motor unit group, amplitude of steady state synaptic depolarization was linearly related to R_N ($r=.64$; $p < .001$; $n=22$). The S and FR samples are thus far too small for similar analysis.

We conclude that R_N is an important determinant of E_m during steady state synaptic depolarization. However E_m during transient synaptic depolarization (i.e. PSPs) may depend upon Z_N and thus upon the spectral components of the synaptic current transient (3, 4). These may be quite variable from one afferent termination to another (5); thus Z_N for a particular afferent termination may not be predictable on the basis of R_N.

References: (1) Fleshman, et al., J. Neurophysiol. 46; (2) Friedman, et al., ibid.; (3) Rall, ibid. 30; (4) Redman, J. Physiol. 234; (5) Jack, Miller et al., ibid. 215.

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225.9 VOLTAGE REGULATED Ca^{++}-DEPENDENT DOPAMINE RELEASE FROM STRIATAL SYNAPTOSOMES USING HPLC/ELECTROCHEMICAL DETECTION. M.G. Hamilton*, and D.H. Ross (SPON: R.E. Huffman). Div. Molecular Pharmac., Univ. Tex. Hlth. Sci. Ctr., San Antonio, TX 78284

Current techniques for measuring resting or evoked transmitter release from neural tissue suffer multiple drawbacks: 1).Use of exogenous radio-labeled neurotransmitters which may not be stored in releasable transmitter pools; 2) Use of long time periods (2-10 min) to demonstrate significant release; 3) Use of non-physiologic levels of K^+ to induce release (>40 mM) and high concentrations of Ca^{++} (>1 mM) to effect release. Current studies using brain slices can not accurately measure Ca^{++} kinetic requirements due to non-specific Ca^{++} accumulation. We report here a system in which time, $K^+$and Ca^{++} requirements have been selectively identified in P_2 fractions from striatal tissue and coupled to dopamine release. Striatal tissue preparations (P_2 pellet), rich in nerve endings, were prepared from rat brain by conventional centrifugation methods. Tissue preparations were preincubated at 37°C in resting buffer containing (5 mM K^+) in the presence of varying Ca^{++} concentrations (100 μM-2000 μM) for 10 min. Ca^{++} influx was initiated by addition of the protein to various tubes containing 5-36 mM K^+ buffers containing $^{45}Ca^{++}$ (2.5×10^5 cpm/tube). The reaction was quenched at different times (1-120 sec) by dilution with ice-cold resting buffer containing 3 mM EGTA, then filtered over Millipore filters (.45 μ) and washed with ice-cold resting buffer (3 times) containing 2 mM $CaCl_2$ (-EGTA). Filters were dried, counted and Ca^{++} influx expressed as nmoles/mg/sec. To determine dopamine release aliquots of the same protein suspension were treated in identical fashion as above except the filtrate was collected in tubes containing 100 μl 0.04 N $HClO_4$. Aliquots of the acidified filtrate were injected directly into an HPLC equipped with a 5 μM octadecylsilyl column and electrochemical detection. Voltage-induced (25 mM K^+) Ca^{++} influx exhibited V_{max} of 537 pmoles/mg/sec and K_m of .780 mM. Ca^{++} dependent dopamine release at saturating Ca^{++} influx increased with increasing K^+ from 40 pg/5 sec at 12 mM K^+ to 166 pg/5 sec at 36 mM K^+. The majority (>70%) release occurred within 15 sec. The rates of release at 25 mM K^+ at 15 sec (44 pg/sec) decreased to 8.5 pg/sec at 120 sec.

These studies demonstrate that voltage-dependent Ca^{++}-stimulated dopamine release from striatal nerve endings may be monitored by HPLC in a system simultaneously measuring depolarization-dependent Ca^{++} influx and transmitter release. Further, this study uses P_2 tissue preparations under more physiological assay conditions than those used previously.

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225.10 CALCIUM-INDEPENDENT RELEASE OF ENDOGONOUS ASPARTATE, GLUTAMATE AND -AMINOBUTYRIC ACID FROM TISSUE SLICES OF RAT VISUAL CORTEX IN VERATRIDINE-, LITHIUM- OR CHOLINE-CONTAINING MEDIUM. Robert W. Baughman and Janice K. Ryu*. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Aspartate (Asp), glutamate (Glu) and -aminobutyric acid (Gaba) are possible neurotransmitters in cerebral cortex. Previously we reported that release of these compounds from tissue slices of rat visual cortex increases manyfold in elevated K+ or veratridine-containing medium (Baughman and Gilbert, J. Neurosci. 1:427 (1981)). The release in high K+ was blocked in low-Ca++ medium and the veratridine-induced release was blocked by tetrodotoxin. We have now found that the veratridine-induced release is independent of extracellular Ca++. Release of Asp, Glu and Gaba increased 5.3X, 10.5X and 19.5X respectively in 10 μM veratridine, and 5.8X, 13X and 24X in 10 μM veratridine with low Ca++ (0.1mM Ca++, 10mM Mg++). In this and all following experiments the release of 12 other amino acids was unchanged. Veratridine presumably increases Na+ influx, which suggests that this release is mediated by a Na+-dependent mechanism. One possibility is the high-affinity transport system for these amino acids. To test this slices were incubated in medium with Li+ substituted for Na+ (veratridine was no longer present) which should block the high-affinity transport mechanism. In fact the release was not blocked (Asp increased 13.5X, Glu 20X and Gaba 18X) and again was independent of extracellular Ca++. Another alternative is that the influx of Na+ or Li+ (Li+ can enter through voltage-dependent Na+ channels) may have some intracellular effect, such as releasing Ca++ from internal stores, which then leads to release. The Na+ in the medium was therefore replaced with choline, an impermeant cation. Release of Asp and Glu was blocked in this medium and Gaba release was reduced to 5.8X control. The remaining Gaba release was independent of extracellular Ca++. These results show that Asp, Glu and Gaba can be released from cortical tissue slices in a manner independent of extracellular Ca++,but that such release, particularly for Asp and Glu, may require the influx of an ion such as Na+ or Li+. (Supported by NIH Grant EY03502 and the Sloan Foundation).

225.11 EFFECTS OF EGTA ON NEUROTRANSMITTER RELEASE IN SYNAPTOSOMES. C. Arias*, M. Sitges* and R. Tapia (SPON: R. Salceda). Dept. de Neurociencias, Centro de Investigaciones en Fisiología Celular, Univ. Nal. Autón. México, 04510-México, D.F., México.

EGTA [ethyleneglycol bis(aminoethylether)tetraacetate] is widely used to chelate calcium ions in studies of transmitter release. In the present communication we report that in mouse brain synaptosomes continuously superfused, EGTA at concentrations as low as 50 μM induces a notable increase of [^{3}H] GABA release (200%-300%) in a medium containing neither Mg^{2+} nor Ca^{2+}. EGTA in excess concentration with respect to these two cations produced a similar stimulation of the release of labeled GABA. This effect of EGTA was strictly dependent on the presence of Na^+ in the superfusion medium, since it was abolished when NaCl was substituted by isosmotic sucrose or by LiCl, but it was not modified when sodium acetate replaced NaCl in the medium. A dose-response curve was obtained when Na^+ concentrations were varied. Under the same experimental conditions in which EGTA greatly stimulated [^{3}H] GABA release, this chelating agent had no effects at all on the release of labeled acetylcholine in synaptosomes previously loaded with [^{3}H] choline. These results suggested that the Na^+-dependent release of GABA produced by EGTA could be due to a carrier-mediated outward transport of this amino acid, which has been described in synaptosomes under conditions inducing Na^+ inward movement. This possibility was tested by using 2,4-diaminobutyrate, which has been demonstrated to block the Na^+-dependent carrier-mediated GABA transport. This compound failed to inhibit the EGTA-induced release of labeled GABA, whereas it greatly reduced the release produced by unlabeled GABA through the well known Na^+-dependent homoexchange mechanism. We conclude that free EGTA, but not the EGTA-Mg or EGTA-Ca complexes, is able to induce an entrance of Na^+, possibly because it chelates calcium ions bound externally to the synaptosomal membrane, and that this entrance enhances the release of labeled GABA by a mechanism to which the release of acetylcholine is insensitive. This mechanism does not seem to be the carrier-mediated transport of GABA. The results obtained indicate that EGTA should be used with caution in studies of the Ca^{2+}-dependence of the release of neurotransmitters, especially at concentrations higher than those of Mg^{2+}. On the other hand, EGTA in the absence of Ca^{2+} and Mg^{2+} seems to be an interesting tool to study the role of Na^+ in the release of neurotransmitters.

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225.12 EFFECTS OF LANTHANUM AND 4-AMINOPYRIDINE ON THE BINDING OF RUTHENIUM RED TO SYNAPTOSOMES. R. Tapia and E. Morales*. Dept. de Neurociencias, Centro de Investigaciones en Fisiología Celular, Univ. Nal. Autón. México, 04510-México D.F., México.

Ruthenium red (RuR) is an inorganic dye which has been shown to inhibit Ca^{2+} binding in CNS membranes and to block the Ca^{2+}-dependent release of neurotransmitters in neuromuscular junctions and in synaptosomes from mouse brain. RuR injected intracranially produces convulsions, whereas administered intraperitoneally causes severe flaccid paralysis. We have found that the RuR-induced flaccid paralysis in mice is antagonized by the injection of 4-aminopyridine (4-AP) during paralysis, and by La^{3+} but only when this cation was administered prior to RuR injection. In the present communication we have studied spectrophotometrically the effects of both 4-AP and La^{3+} on the binding of RuR to synaptosomes from mouse brain. It was observed that the binding of RuR was extremely rapid, and about two-fold greater in the absence of cations (sucrose-Tris medium) than in their presence (Krebs-Tris medium). La^{3+} preincubated with the synaptosomes at 100 μM concentration notably inhibited RuR binding. In sucrose-Tris the bound RuR was not released by resuspension of the synaptosomes, and when La^{3+} was present in the resuspension medium 6-8% of the bound dye was displaced. In Krebs-Tris medium about 25% of the dye was released by resuspension and this value was increased to 40% when 100 uM La^{3+} was present in the resuspension medium, both in the absence and in the presence of 2 mM Ca^{2+}. In contrast to these results, 0.2 mM 4-AP did not displace RuR from the synaptosomes in a Krebs-Tris medium. It is concluded that RuR and La^{3+} share at least one kind of site on the synaptosomal membrane, and that the inhibition of RuR binding by La^{3+} might be important for the antagonism of their effects in vivo. On the other hand, the antagonist action of 4-AP on RuR-induced paralysis in vivo must be due to a different mechanism, probably its stimulatory action on transmitter release.

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225.13 SPECIFIC CYTOTOXICITY AND BINDING OF ß-BUNGAROTOXIN IN CHICK CNS. H.A. Rehm* and H. Betz*. (SPON: ENA). Max-Planck-Institute for Psychiatry, Department of Neurochemistry, 8033 Martinsried, Federal Republic of Germany.

ß-Bungarotoxin (ß-BTX) is a presynaptically active, Ca^{++}-dependent phospholipase A_2 isolated from the venom of the snake Bungarus multicinctus. Using organ cultures of chick retina, the toxin's cytotoxicity was investigated by monitoring the loss of different marker enzymes. It was found that ß-BTX is active at picomolar concentrations and specific for neurons. The toxin furthermore displayed selectivity between neuron subclasses, preferring cholinergic and GABAergic to dopaminergic neurons. Histology showed that only neurons in the ganglion and amacrine cell layers were destroyed. This selective cytotoxicity of ß-BTX could not be mimicked by the non-neurotoxic phospholipases A_2 from bee venom or porcine pancreas.

In order to explore whether the selective cytotoxicity of ß-BTX might be due to the existence of specific binding sites, the binding of ^{125}I-labeled ß-BTX to synaptic membrane fractions of chick brain was investigated. A single class of high affinity binding sites (K_D = 0.47±0.14 nM) was found, whose pharmacological properties correlated with the cytotoxicity of ß-BTX. The density of ^{125}I-ß-BTX binding sites in synaptic membrane fractions was low (50 fmol/mg protein). Specific toxin binding was restricted to tissues known to contain ß-BTX sensitive cells or nerve endings, i.e. there was binding to membrane fractions from brain, retina and muscle, but not from liver or heart. The binding of ^{125}I-ß-BTX was dependent on Ca^{++}. This ion could be replaced by Co^{++} or Sr^{++}, but not by Mg^{++}. The membrane binding site for ^{125}I-ß-BTX was sensitive to heat and high concentrations of pronase and thus most likely is a protein.

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225.14 **β-BUNGAROTOXIN INHIBITS SYNAPTOSOMAL CALCIUM UPTAKE.** **Gene S. Tobias.** Southern California College of Optometry, Fullerton, CA 92631.

β-bungarotoxin has inherent phospholipase A enzymatic activity and acts presynaptically to inhibit both evoked and spontaneous acetylcholine release in a triphasic pattern with time after toxin application. In the first phase there is a transient decrease in release which has been ascribed to an inhibition of Ca^{++} entry into the presynaptic ending. The phospholipase activity of the toxin is not required for this initial phase effect. The enzymatic activity of the toxin is necessary for the second phase of supernormal release and for the third phase of decreasing and ultimate blockage of release, and it has been proposed that these latter phase effects may be due to increased Ca^{++} entry into the presynaptic ending. To determine whether β-bungarotoxin may act by alternately inhibiting and enhancing Ca^{++} entry as proposed to yield the triphasic response, $^{45}Ca^{++}$ uptake into rat brain synaptosomes was assayed, using $^{45}Ca^{++}$ as a tracer, 15 and 60 minutes after toxin application; i.e., during the first and second phases, respectively, of toxin action. Synaptosomes were incubated in 5 or 50 mM K^+ to mimic the conditions of spontaneous or evoked release, respectively, and with 10 μg/ml enzymatically active or inactive β-bungarotoxin (enzymatically inactive toxin was produced by heat treatment which reduced the phospholipase activity by 95%). Neither toxin had any effect on Ca^{++} uptake into synaptosomes in 5 mM K^+ at the 15-minute exposure time, but both toxins inhibited the increased Ca^{++} uptake induced by 50 mM K^+ by 50% at this early exposure time. At the 60-minute exposure time enzymatically inactive toxin had no effect on uptake in 5 mM K^+ but inhibited the increased Ca^{++} uptake in 50 mM K^+ by 90%. Enzymatically active β-bungarotoxin on the other hand caused a 50% increase in Ca^{++} uptake in low K^+; whereas, this toxin had no effect on the increased uptake in high K^+. These results generally support the hypothesis that for evoked release the triphasic pattern which occurs over time after toxin application is caused by β-bungarotoxin's effect on Ca^{++} entry into the presynaptic ending. However the results under the spontaneous release conditions suggest that the toxin may be acting by a mechanism independent of Ca^{++} entry.

225.15 BIOLOGICAL SIMILARITY OF TWO PHOSPHOPROTEINS (PROTEINS IIIa AND IIIb) ASSOCIATED WITH SYNAPTIC VESICLES IN RAT BRAIN. M.D. Browning, C-K. Huang*, and P. Greengard. Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

We have recently shown that depolarization of brain slices in the presence of calcium or bath application of 8-bromo-cAMP to brain slices produced an increase in the phosphorylation of four acid soluble proteins (Proteins Ia and Ib, M_r 86,000 and 80,000, respectively; Proteins IIIa and IIIb, M_r 74,000 and 55,000, respectively). We recently described the partial purification and characterization of Protein IIIb. We now report that Proteins IIIa and IIIb possess significant biological similarities. The two proteins copurify, exhibiting solubility at pH 3.0 and co-elution upon ion exchange and hydroxylapatite chromatography. Two-dimensional isoelectric focusing revealed that Protein IIIa yields multiple spots ranging in pI from 6.8-7.1 and Protein IIIb yields multiple spots ranging in pI from 6.6-7.0. Both proteins can be phosphorylated by the catalytic subunit of cAMP-dependent protein kinase. Moreover, one-dimensional phosphopeptide maps of the two proteins are essentially identical. Antibodies raised in rabbits against homogeneous denatured Protein IIIb revealed 100% cross-reactivity with Protein IIIa. These antibodies also exhibited mild cross-reactivity (≤10%) with Protein I. Seven monoclonal antibodies raised against homogeneous Protein IIIb exhibited 100% cross-reactivity with Protein IIIa, while partial cross-reactivity toward Protein I was seen with some but not all of these monoclonal antibodies. We have studied the tissue distribution of Proteins IIIa and IIIb with a radioimmunoassay that is sensitive to as little as 10 femtomoles of either Protein IIIa or IIIb, and we have been unable to detect any competition from 100 μg of homogenates of liver, kidney, spleen, or heart. We have used a Protein A immunolabeling technique to estimate the amounts of Proteins IIIa and IIIb in various subcellular fractions of rat brain. The data reveal that the two proteins have parallel subcellular distributions and that synaptic vesicle fractions contain the highest concentrations of the two proteins. The data suggest that Proteins IIIa and IIIb have similar roles in the brain, and that they may be involved in regulation of synaptic vesicle function.

225.16 THE SELECTIVE ALTERATION OF NEUROTRANSMITTER RELEASE BY ANOXIA Joseph A. Hirsch* and Gary E. Gibson. Cornell Medical College, Burke Rehabilitation Center, White Plains, New York 10605.

Hypoxia (i.e. decreased oxygen availability) impairs cognition (Brain Energy Metabolism, B. Siesjö, 1978) by unknown molecular mechanisms. The in vitro calcium-dependent potassium-stimulated release of acetylcholine from rat brain slices or synaptosomes decreases during anoxia (absence of oxygen), whereas norepinephrine release is unaffected (Gibson and Peterson, Fed. Proc. 65:199, 1981; Hirsch and Gibson, Fed. Proc., 66:8738, 1982). We have now extended those studies to examine the effects of anoxia on the release of serotonin and glutamate.

Release of either tritiated serotonin from prelabelled stores or endogenous glutamate was evoked by superfusion of hand-cut slices from rat cerebral cortex with 50 mM-KCl. Neurotransmitter release was determined with 95% (normoxic) or 0% oxygen (anoxic) Krebs-bicarbonate buffer with or without 2.0 mM-calcium. Anoxia did not alter the fractional release of tritiated serotonin in the presence (normoxia = 0.240 ± 0.013; anoxia = 0.275 ± 0.020) or absence of calcium (normoxia = 0.007 ± 0.006; anoxia = 0.004 ± 0.003). These negative findings parallel those that we reported previously for norepinephrine. Anoxia enhanced the potassium-evoked release of glutamate in the presence of calcium from 2.44 ± 1.71 to 12.23 ± 2.51 nmole/mg protein (n = 5). Release in the absence of calcium was not consistently altered by anoxia. Thus, the release of two glucose-derived neurotransmitters - acetylcholine and glutamate - is modified by anoxia.

The selective alteration of the release of these various neurotransmitters by anoxia suggests that their release mechanisms differ. The alterations in acetylcholine and glutamate release by anoxia may underlie the cognitive deficits that accompany mild hypoxia or the siezures that occur during severe hypoxia.

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225.17 BRAIN PYRUVATE DEHYDROGENASE: SUBCELLULAR COMPARTMENTALIZATION OF FUNCTIONAL REGULATION. R. Akers*, S.I. Cain*, and A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, Il. 60201.

The α-subunit of brain pyruvate dehydrogenase (PDH) has been implicated in various forms of neural plasticity (Morgan and Routtenberg, Science, 1981; Browning et.al., Science, 1979). These studies have not examined from which neuronal compartments these alterations in PDH activity and phosphorylation were found. Differences in the activation state of PDH have been observed in enzyme derived from synaptosomal vs. "free" mitochondria (Deshmukh et.al., J. Neurochem., 1979); differences may also exist in the regulation of PDH from these two fractions.

We have analyzed PDH activity and phosphorylation in subcellular fractions of myelin (1), synaptosomes (2), and free mitochondria (3) (Hajos, Br.Res., 1975) isolated from rats sacrificed by decapitation, and homogenized rapidly (within 30 seconds) in ice-cold 0.32M sucrose. A phosphoprotein of MW 80,000 (termed Band D, or Protein I) which has been localized primarily in presynaptic terminals, is highly enriched in our synaptosomal fractions. PDH activity and phosphorylation were determined (Morgan and Routtenberg, Science, 1981). A back-titration phosphorylation assay was performed by incubating brain fractions with 1 mM [32-P]-ATP and 1 mM EDTA. Mg++ chelation prevents PDH phosphatase, but not kinase, activity, and vacant sites of phosphorylation can be estimated.

Total PDH activites (nmol CO_2 evolved per min per mg protein) were: 1.±.07 (1); 12.18±.47 (2); 28.55 ±.08 (3). It was found that PDH phosphorylation in (3) was greatly enhanced in animals sacrificed by whole body immersion in liquid nitrogen.

Functional manipulations *in vivo* which influence PDH activity, such as barbituate anesthesia and food deprivation, have differential effects on PDH activity in fractions (2) and (3). PDH derived from presynaptic terminals may thus be regulated differently from predominantly postsynaptic PDH. Supported by N.I.M.H. 25281 to A.R.

226.1 TWO CONDUCTANCES ACTIVATED BY EXCITATORY AMINO ACIDS, J.F. MacDonald and A.V. Porietis. Playfair Neuroscience Unit, Dept. Pharmacol., Univ. Toronto, Toronto, Ontario M5T 2S8.

Excitatory amino acids are believed to depolarize central mammalian neurons by increasing G_{Na^+} (Na^+ conductance) and possibly $G_{Ca^{2+}}$. However, measurements of input conductance (G_M) have provided evidence of both increased or decreased G_M in the presence of such amino acids. We have attempted to clarify the mechanisms of excitation by these compounds using mouse spinal cord neurons grown in tissue culture. One or two intracellular electrodes were used in current and/or voltage-clamp of individual neurons. Two distinct mechanisms of excitation have been identified. The first is preferentially activated by amino acids such as DL-kainic, DL-quisqualic and D-homocysteic and is manifest as an inward current under voltage-clamp. It reverses to an outward current at -10 mV and varies in magnitude in a manner consistent with changes in driving force on the ions likely involved. A second mechanism is activated to varying degrees by amino acids such as L-aspartic, L-glutamic and L-homocysteic. Inward currents are also evoked with a reversal potential co-incident to that of the first mechanism. However, hyperpolarization from -60 mV gradually reduces this current to nil at values between -80 to -90 mV despite an anticipated increase in driving force. This deviation demonstrates the inactivation of a highly voltage-dependent inward current. This voltage- dependent mechanism was selectively blocked by Cd^{2+} (0.5 and 1.0 mM) but was insensitive to putative Ca^{2+} antagonists such as verapamil and D-600 and to perfusion with low Ca^{2+} bathing solutions. Total substitution of Na^+ with choline or tris shifted the reversal potential (of both mechanisms) to values near resting and gradually eliminated conductance changes suggesting a primary involvement of G_{Na^+}. However, an occasional increase in spontaneous "synaptic-like" activity, despite the presence of tetrodotoxin, also implied a secondary elevation of intracellular Ca^{2+} secondary to the removal of extracellular Na^+ and the possible reduction of the drving force on any putative inward Ca^{2+} flux. Substitution with Li^+ did not always reduce the amino acid-induced conductance change and often accentuated the voltage-dependent mechanism suggesting that the channel involved may also be permeant to this ion. Likely, this channel has a poor selectivity for cations. The activation of this voltage-dependent mechanism accounts for apparent measurements of decreased G_M in current-clamp, the amino acid-induced negative slope conductance in voltage-clamp and the regenerative "bursting" recorded in the presence of certain amino acids.

Supported by M.R.C.

226.2 AMINO ACID ACTIVATED CALCIUM CONDUCTANCE IN HIPPOCAMPAL PYRAMIDAL CELLS. Raymond Dingledine. Dept. Pharmacol., Univ. N. Carolina, Chapel Hill, NC 27514.

Multiple receptors for excitatory amino acids are known to exist on mammalian neurons. The most well defined receptor is selectively activated by N-methyl-D-aspartate, and is termed an NMDA receptor. The basis for the depolarizing actions of the NMDA receptor agonist N-methyl-DL-aspartate (NMA), and glutamate, were compared in an in vitro hippocampal slice preparation bathed in 1 μM tetrodotoxin to suppress sodium spikes. Intracellular recordings were made from pyramidal-type neurons; agonists were applied by iontophoresis while antagonists were applied by perfusion or in a droplet of bathing medium onto the slice surface. Iontophoresis of NMA triggered slow, high threshold spikes that could be blocked by verapamil, D-600, Co++, Mn++ and Cd++, and potentiated by Ba++, and are thus considered to be calcium spikes. Depolarizations evoked by glutamate only rarely triggered calcium spikes and were usually associated with a fall in input resistance. NMA caused an apparent increase in input resistance. This is not considered to reflect an actual decrease in resting potassium conductance, but rather a rise in a voltage sensitive calcium conductance, for the following reasons: First, depolarizations and conductance changes elicited by NMA could be completely blocked by Co++, Mn++ and Cd++, and reduced by verapamil and D-600. These calcium antagonists had little or no effect on resting membrane potential, input resistance, or glutamate responses. Ba++ potentiated NMA response. Second, raising extracellular [K] from 3.5 to 10.5 mM did not affect NMA responses. Reducing extracellular [Na] by 86% caused an initial increase, and then a delayed decrease in the amplitude of the NMA response; this is expected if Na/Ca exchange were impaired in low Na, leading to a rise in intracellular [Ca]. Low Na also reduced the amplitude of calcium spikes evoked by depolarizing current pulses. Third, the depolarization and conductance change evoked by NMA were voltage dependent. Both could be abolished by hyperpolarizing the cell with steady current injection to -70 to -90 mV, but no reversal potential could be demonstrated. The minimum latency to the onset of NMA-evoked depolarization was <20 ms. NMDA receptors on hippocampal pyramidal cells are thus linked to voltage sensitive calcium channels. Since certain forms of synaptic plasticity in the hippocampus appear sensitive to NMDA receptor antagonists, a postsynaptic rise in calcium conductance may be involved in synaptic plasticity. Supported in part by the Sloan Foundation and NIDA Grant DA-02360.

226.3 APPARENT SENSITIZATION OF GABA-INDUCED RESPONSES IN HIPPOCAMPAL NEURONS. F.J. Lebeda & J.J. Hablitz, Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030

The study of transmitter-receptor stoichiometry at central inhibitory synapses is essential in characterizing the actions of drugs (e.g. convulsants and anticonvulsants) which may exert their effects at these sites. In the course of examining responses induced by iontophoresis of gamma-aminobutyric (GABA) onto CA3 neurons in guinea pig hippocampal slice preparations, extensive variability in the stoichiometry was encountered (Lebeda et al., J. Neurophysiol., in press). Although GABA consistently produced maximum reductions in input resistance (Rin; 80-90%), a wide range of slopes (1.5-8.5) for the charge-response curves was obtained. Since the slope value for a given neuron was stable with time, we reasoned that despite the use of retaining currents, leakage of GABA may have produced this variability among cells. To test this idea, we initially used GABA-filled, double barrel pipettes to provide a preset background of excess agonist.

Maintained iontophoresis of GABA (5 min) from one barrel (at a level which did not appreciably affect the resting potential or Rin) produced a leftward, nonparallel shift in the charge-response curve. The submaximal responses to short pulses of GABA were increased and prolonged, while the slope became markedly decreased. When larger amounts of GABA were continuously applied, a sustained decrease in Rin along with a less extensive leftward shift, and a depression of some of the larger GABA-mediated responses occurred. To determine if the reuptake of GABA was involved with these effects on the charge-response relation, the reuptake antagonist, nipecotic acid (Nip; 0.5 M; pH 4.0), was continually iontophoresed from the second barrel. With low ejection currents, Nip caused a sensitizing effect similar to that obtained with excess GABA. Larger amounts of ejected Nip produced a decrease in Rin and a depression of some of the GABA-mediated responses.

Finally, a kinetic model was formulated which simulated the decrease in slope and the apparent sensitization of the agonist-induced responses. This was achieved by having two classes of agonist binding sites: one with, and the other without, ionic channels. Apparent sensitization resulted when the latter sites were reduced in number or became saturated with the agonist.

It is hypothesized that the variability in the charge-response curves is related to differing amounts of GABA leaking from iontophoretic pipettes which in turn affect binding sites not associated with GABA-activated ionic channels, perhaps GABA reuptake sites.

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226.4 THE STIMULATION OF $^{36}Cl^-$ FLUX IN RAT BRAIN SLICES BY γ-AMINOBUTYRIC ACID (GABA). E.H.F. Wong*, L.M.F. Leeb-Lundberg,* V.I. Teichberg and R.W. Olsen. University of California, Riverside, CA, 92521.

γ-Aminobutyric acid (GABA) and other GABA receptor agonists increase the efflux rate of $^{36}Cl^-$ from preloaded rat hippocampal slices in a picrotoxinin- and bicuculline-sensitive manner, suggesting an interaction with a functional postsynaptic GABA receptor. The method used was based on that of Luini et al., PNAS 78, 3250-3254 (1981). Fresh rat hippocampal slices (250 μm) were incubated for 40 min in oxygenated physiological Ringer's medium at 37°C, and then loaded with 2 μCi/ml of $^{36}Cl^-$ for 30 min. Efflux was measured by transferring them every minute through a series of tubes containing nonradioactive medium. After a constant efflux rate of $^{36}Cl^-$ was reached (15 min), introduction of GABA or the potent GABA-mimetic, muscimol, resulted in a significant increase in $^{36}Cl^-$ efflux rate. The response to GABA was saturable and dose-dependent with an EC_{50} of 0.4 mM. Muscimol was approximately 5-10 times more potent. The effects of both agonists were blocked 50% by 10-100 μM picrotoxinin and bicuculline, known GABA antagonists. Similar results were obtained in slices from striatum or frontal cortex. GABA also stimulated the efflux of $^{125}I^-$ but not $^{22}Na^+$, as expected for ion-channels associated with inhibitory synaptic transmission. Furthermore, glutamate did not increase the efflux of $^{36}Cl^-$. These results strongly suggest that GABA-stimulated $^{36}Cl^-$ efflux involves postsynaptic receptor-ionophore function. The brain slice system provides a convenient assay for the study of GABA receptor function and its modulation by various depressant and excitatory drugs.

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226.5 IS THE LATE HYPERPOLARIZATION WHICH FOLLOWS SYNAPTIC STIMULATION OF HIPPOCAMPAL PYRAMIDAL NEURONS CALCIUM-DEPENDENT? R.H. Thalmann, Dept. of Cell Biology and Program in Neuroscience, Baylor College of Medicine, Houston, Texas 77030

After moderate or low level synaptic stimulation of several neuronal types in the hippocampus, an increased potassium (K) conductance follows the familiar GABA-mediated IPSP. Several experiments have been performed to test the hypothesis that this potassium conductance (the late hyperpolarization or LH) is calcium (Ca)-dependent. Intracellular recordings were made from neurons in the CA3 region of the hippocampal slice, maintained by standard methods. In one series of experiments, the recording electrodes contained 0.25-0.5 M EGTA, a Ca chelator, in addition to the K-acetate electrolyte; the EGTA was allowed to diffuse into the neuron, or was injected with hyperpolarizing current pulses. Stimulation of the mossy fiber pathway was used to elicit the LH. Since mossy fibers are known to synapse with CA3 neurons in a restricted zone near the cell body, it seemed possible that this cell type offered the best chance that EGTA, iontophoresed via the recording electrode, could reach any Ca-dependent mechanism that might be regulated by orthodromic stimulation. Reduction and disappearance of the Ca-dependent K conductance (AHP) which followed a 1-2 Na depolarizing current pulse was used as an assay for the successful injection of EGTA. These procedures have failed to block the LH, despite the fact that the Ca-dependent AHP regularly declines and then disappears within seconds to minutes after impalement with the EGTA-containing electrodes. In an attempt to reduce or block the effects of the EPSP on hypothesized voltage-dependent Ca channels, the membranes of CA3 neurons were hyperpolarized by 45-50 millivolts only during the initial 50 milliseconds of the synaptic events which follow stimulation of the mossy fiber input. This procedure also failed to block the LH. Finally, we have observed the LH in the absence of antecedent depolarizing events which were detectable at the resting membrane potential. It is possible that a synaptically activated Ca-dependent K mechanism is isolated electrically to the extent that voltage-dependent Ca events are not visible to, or subject to effect by, an intrasomatic electrode. It is also possible that the hypothesized Ca channel is not of the voltage-dependent type, and that the intracellular increase in Ca concentration, however rgulated, is compartmentalized in the Ca3 neuron such that EGTA injected at the some cannot affect it. Alternatively, the LH may not be Ca-dependent. Supported by National Institutes of Health, Grant NS-11535.

226.6 NOREPINEPHRINE (NE) AND ADRENERGIC AGONISTS IN LOW CONCENTRATIONS ALTER SPONTANEOUS ACTIVITY AND MEMBRANE POTENTIAL OF CA1 PYRAMIDAL NEURONS OF THE RAT HIPPOCAMPUS IN VITRO. D.L. Gruol and G.R. Siggins. A.V. Davis Center, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Noradrenergic fibers originating from the nucleus locus coeruleus innervate the hippocampus and are believed to regulate hippocampal neuronal activity. We have recorded intracellularly in the in vitro hippocampal slice preparation to investigate the mechanisms by which NE alters the activity of CA1 pyramidal neurons (HPNs). Hippocampal slices (350-400 µm thick; obtained from 90-130 gm rats) were maintained totally submerged under conditions of constant superfusion (1-2 ml/min; oxygenated artifical CSF) during electrophysiological studies. Drugs were applied by superfusion. All 17 HPNs studied displayed stable resting potentials of -55 to -75 mV, spikes of 60 to 80 mV and spontaneous activity consisting of spikes and subthreshold events. Previously we reported that NE and isoproterenol (IP) at high concentrations (50 µM or more) had mixed effects on the activity of HPNs with no consistant change in membrane potential. In the present experiments, low concentrations of NE and IP were tested. At concentrations below 1 µm, NE was relatively ineffective in altering the activity of HPNs (n=6). At 1 to 3 µM in 10 cells NE increased the spontaneous activity of 8 cells, decreased activity in 1 cell and produced a biphasic increase followed by a decrease in 1 cell. The increase in activity was associated with a membrane depolarization whereas the decrease in activity was associated with a small hyperpolarization. The alpha adrenergic agonist clonidine was tested on 6 neurons at concentrations ranging from 0.2 to 5 µM. Clonidine decrease spontaneous activity in 4 cells and had no effect in 2 cells. A small hyperpolarization was associated with the decrease in activity. The beta adrenergic agonist isoproterenol (IP) mimicked the excitatory action of NE but was considerably more potent. IP increased activity and depolarized the neurons (8 cells, 18 trials) in a dose-dependent manner. Effective concentrations of IP were as low as 50 nM in some neurons. IP also reduced the after-hyperpolarization (AHP) generated by current-evoked action potentials. The AHP is thought to be mediated by a Ca++ dependent K+ conductance in HPNs. In CSF containing high Mg++, which blocks Ca++ conductances, the effect of IP on HPNs activity could not be demonstrated. These data suggest that NE can both increase and decrease the activity of HPNs and that these actions may be mediated by β and α adrenergic receptors, respectively. At least some of the actions mediated by the β receptor may involve an alteration of a Ca++ conductance. (Supported by AA03504 and MH 29466)

226.7 INTRACELLULAR CORRELATES OF PAIRED-PULSE POTENTIATION IN HIPPOCAMPAL PYRAMIDAL CELLS: RELATIONSHIP TO AFTERHYPERPOLARIZATION. R.W. Turner*, T.L. Richardson* & J.J. Miller (SPON: A.G. Phillips). Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C. V6T 1W5.

Several studies have demonstrated a potentiation of evoked population responses in the hippocampal formation following conditioning stimulation of afferent inputs to this structure. While the characteristics of paired pulse potentiation have been examined in detail using extracellular potentials, little is known of the intracellular correlates of this phenomenon. In the present study simultaneous intra- and extracellular recordings were carried out in the in vitro hippocampal slice preparation to compare the unitary and field potentials of CA1 pyramidal cells in response to condition-test (C-T) stimulation of stratum radiatum (SR).

Single pulse stimulation of SR evoked a characteristic EPSP-IPSP sequence and a prolonged after-hyperpolarization (AHP) lasting for 500-700 msec. Increasing stimulus intensity resulted in a larger amplitude EPSP followed by a shorter onset latency and longer duration IPSP/AHP response.

At subthreshold intensities, the amplitude of the EPSP evoked by the test stimulus was depressed when preceded by a conditioning pulse at intervals of 0-40 msec. In contrast, with C-T intervals of 40-100 msec. there was an increase in probability of AP discharge. At higher stimulus intensities the duration of this effect was prolonged to 500 msec. The time course of the intracellular events correspond to the relatively short period of inhibition and subsequent potentiation of the extracellular population spike, providing direct evidence that recruitment of silent neurons contributes to paired pulse facilitation. In addition, the results indicate that the period of maximal probability for AP generation occurs during the hyperpolarization, and increases with stimulus intensity despite a parallel increase in the underlying IPSP/AHP potential. These data suggest that a hyperpolarizing process may paradoxically increase somatic sensitivity for AP generation, and as a consequence result in paired pulse potentiation. However the fact that 1) spontaneous activity was inhibited during the AHP, 2) threshold for AP discharge as measured with depolarizing pulses was greater and 3) hyperpolarizing current could block test pulse AP's occurring during the AHP, indicates that the increased probability to discharge cannot be attributed to an increase in somatic sensitivity. Alternatively this effect may be dependent upon mechanisms localized to presynaptic or dendritic regions of pyramidal neurons.

226.8 ARE SLOW IPSPs IN APLYSIA NEURONS MEDIATED BY INTRACELLULAR MESSENGERS? R. H. Kramer* and R. S. Zucker (SPON: E. R. Lewis) Dept. Physiology-Anatomy, Univ. of Calif., Berkeley, CA 94720.

Post-synaptic potentials that have a slow onset and are long-lasting are candidates for mediation by intracellular messengers. We have been investigating the possible roles of Ca^{2+} and cyclic AMP (cAMP) in generating slow IPSPs in cell R15 of the abdominal ganglion and the medial cells of the left pleural ganglion of Aplysia.

R15 has a high threshold synaptic input (input III of I.Parnas and F.Strumwasser, J. Neurophysiol. 37:609-620,1974) which can be elicited by stimulation of the right connective or branchial nerve, and which can inhibit spontaneous firing in R15 from a few minutes to hours. The inhibition is in part due to an increase in K^+ conductance. We have used pressure injection of EGTA to test the role of Ca^{2+} in generating the response. R15's response to input III stimulation (5 sec at 3 Hz) was changed little by raising the intracellular EGTA concentration to about 50mM, and the changes could be attributed to small changes in membrane resistance which occured following injection. We tested the effectiveness of the EGTA by examining its effects on spike shape and Ca^{2+}-dependent hyperpolarizations following trains of spikes. EGTA injection resulted in an initial depolarization followed by spontaneous bursts of action potentials terminating in a prolonged (up to 5 sec) depolarizing shift. A 7 second train of spikes at 3 Hz was initially followed by a large, slowly decaying hyperpolarization. After EGTA injection, the hyperpolarizing tail was almost completely eliminated.

The role of cAMP in mediating the response was tested by addiag up to 25 µM of the cAMP-phosphodiesterase inhibitor RO-20-1724 to the bathing medium. At high concentrations, a small hyperpolarization from resting potential resulted, but there was no augmentation of the response to input III.

Similar results were obtained for slow inhibitory responses in the medial cells. Long-lasting hyperpolarizations (20 sec-1 min) due to a K^+ conductance increase were elicited by short (<1 sec) iontophoretic pulses of acetylcholine directly onto cell bodies. Changes in the responses due to EGTA injections were entirely attributable to changes in membrane resistance, and were paralleled by changes in rapid, increased Cl^-- conductance inhibitory responses to acetylcholine in the same cell. The EGTA effectively blocked hyperpolarizing tails follwing trains of spikes. Mediation by cAMP was tested by applying RO-20-1724; it had little effect on membrane potential or the respnse to acetylcholine. It does not appear likely that Ca^{2+} or cAMP function as intracellular messengers for the slow IPSPs in either R15 or the medial cells.

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226.9 USE-DEPENDENT CHANGES IN INITIATION SITE AND CONDUCTION VELOCITY OF SPIKES IN APLYSIA NEURONS, Rafiq Waziri and Richard Mooney, Dept. of Psychiatry, Univ. of Iowa School of Med., Iowa City, IA

The propagation times of spikes during repetitive firing was studied in two cells of the Aplysia abdominal ganglion, the giant cell R_2 and the multiaction interneuron L_{10}. Spike propagation in R_2 was measured by simultaneous intracellular recording from the soma and from the main axon at sites 1-3 cm away. Spike propagation in L_{10} was measured by recording from the L_{10} soma and from the soma of L_{20}, which receives an electrical synapse from one of L_{10}'s processes.

When R_2 or L_{10} fires a burst of spikes, a slow depolarization occurs in the axon throughout the burst. As the axon depolarizes, the difference in spike times measured at the soma and in the axon decreases. The amount of this reduction depends on the firing frequency and duration of the burst and also on the conduction distance. A prolonged burst of spikes over a conduction distance of 10 mm, for example, may reduce latencies by 50%.

The basis of this latency reduction appears to be a distal shift in the spike initiation zone, which may be as large as 3 mm in R_2. Two factors that do not contribute to the reduction in axon spike latency are: 1. the conduction velocity, which is actually slower during the period of firing as determined by conduction times of antidromic spikes and 2. the space constant of the axon, which is reduced during firing, and thus, the longitudinal spread of current diminishes. The steady depolarization during firing appears to contribute to inactivation of the most sensitive trigger regions, and it may result from an extracellular build up of potassium ions during the burst. If K^+ concentration in the sea water is raised, the slow depolarization is diminished or absent. In addition, if depolarizing current is injected into the soma, spike latencies in the axon are reduced.

Since maintained firing rates of Aplysia neurons are typically below 10/sec, even large changes in spike conduction times do not appreciably alter spike frequency, but a mobile firing zone may allow continued firing after the initial segment has become fatigued or inactivated; and it would alter the relative timing of spikes entering processes that branch off the main axon in the region through which the firing zone moves.

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226.10 AMMONIA, POSTSYNAPTIC INHIBITION AND CNS ENERGY STATE. W. Raabe and S. Lin*. Depts. Neurology, VA Med. Ctr. and Univ. of Minneapolis, Minneapolis, MN 55417.

Ammonia intoxication is known to cause encephalopathy without changing the gross energy state of the CNS-tissue. This study investigates whether an effect of ammonia on postsynaptic inhibition occurs without a change of the gross CNS energy state and, therefore, qualifies as a cause of ammonia induced encephalopathy. Ammonia inactivates the extrusion of Cl^- from neurons and shifts the equilibrium potential of the IPSP, E_{IPSP}, toward the level of the resting membrane potential. Thus, ammonia abolishes the hyperpolarizing action of IPSPs and their ability to suppress neuronal excitation.

Cats were decerebrated and respirated artificially. Intracellular records of IPSPs were obtained from spinal motoneurons. Ammonium acetate was given slowly i.v. until the E_{IPSP} shifted toward the resting membrane potential. Ammonium acetate infusion was then terminated and the E_{IPSP} allowed to recover. The resting membrane potential showed no significant changes during ammonium acetate infusion. To indicate the inactivation of Cl^- extrusion, the shift of the E_{IPSP} to the resting membrane potential had to occur without changes of the slope of IPSP size vs. membrane potential during applied current steps. The lumbar spinal cord was frozen in situ with liquid nitrogen before (N=4), during (N=4) and after recovery (N=4) of the changes of the E_{IPSP}. Ammonia, glutamate, glutamine, ATP, ADP, AMP, phosphorylcreatine, glucose, pyruvate and lactate were determined.

To shift the E_{IPSP} 2.43 mMol/kg of ammonium acetate had to be infused. With the shift of E_{IPSP} ammonia increased from 0.05 µMol/g to 1.25 µMol/g ($p<0.005$), glutamine increased from 5.64 µMol/g to 10.5 µMol/g ($p<0.05$) and lactate increased from 2.61 µMol/g to 6.69 µMol/g ($p<0.02$). Notably absent were changes of ATP, ADP, AMP, the adenylate energy charge, phosphorylcreatine, glucose and pyruvate with the shift of E_{IPSP}. The changes of ammonia, glutamine and lactate significantly reversed with the recovery of E_{IPSP}.

Ammonia intoxication affects the extrusion of Cl^- from neurons and the hyperpolarizing action of IPSPs without a change of the gross energy state of CNS-tissue. Therefore, the effect of ammonia on Cl^- extrusion and inhibition qualifies as a cause of the encephalopathy produced by ammonia intoxication. The effect of ammonia on Cl^- extrusion abolishes the suppression of neuronal excitation by postsynaptic inhibition (J. Neurophysiol., 38:347, 1975). The resulting dysfunction of inhibitory neuronal interactions can be postulated to cause or contribute to the encephalopathy due to ammonia intoxication.

227.1 DIFFERENCES IN THE REGULATION OF ACETYLCHOLINE SENSITIVITY AND α-BUNGAROTOXIN BINDING ON CHICK CILIARY GANGLION NEURONS IN CELL CULTURE. Martin A. Smith*, Joseph F. Margiotta*, & Darwin K. Berg. Dept. of Biology, Univ. of Calif., S.D.; La Jolla, CA. 92093.

Chick ciliary ganglion neurons have nicotinic acetylcholine (ACh) receptors and high affinity α-bungarotoxin (α-Bgt) binding sites. The relationship between the two is unclear since α-Bgt does not block the sensitivity of the neurons to ACh, and recent ultrastructural findings show that α-Bgt binding sites are near but not part of synapses on the neurons. We now report that culture conditions which regulate growth and development of the neurons have differential effects on the levels of ACh sensitivity and α-Bgt binding.

Ciliary ganglion neurons can be maintained for weeks in dissociated cell culture in a simple medium containing only MEM and 10% horse serum if the K^+ concentration is elevated to 25 mM (K^+ medium). Previous studies have shown that supplementing K^+ medium with extract from eye tissue (K^+/eye medium), which is the normal synaptic target of the neurons, causes a 2-4 fold stimulation in the levels of choline acetyltransferase activity and total cytoplasmic growth. Eye extract alone (eye medium) enhances growth and development nearly 2 fold over K^+ medium. Neuronal survival is similar in the 3 culture media.

Very different results were obtained when two membrane properties, ACh sensitivity and α-Bgt binding, were measured for the neurons after 1 week. ACh sensitivities were comparable for neurons grown in K^+ and K^+/eye media, and were about 3 times higher for neurons grown in eye medium. In contrast, the numbers of α-Bgt binding sites were comparable in eye and K^+/eye media, but were at least 2 times higher in K^+ medium. α-Bgt binding was measured with (^{125}I)α-Bgt and gamma counting. ACh sensitivity was measured with intracellular recording while pressure ejecting ACh from a nearby microelectrode. Neurons were perfused with MEM + horse serum and hyperpolarized to -80 mV for the tests; small corrections were made for delayed rectification and for differences in input resistance among the neurons.

The fact that ACh sensitivity and α-Bgt binding can vary independently suggests that the two represent separate membrane components. Eye extract depresses the levels of α-Bgt binding while 25 mM K^+ partially blocks the expression of ACh sensitivity. Both membrane properties appear to differ in their patterns of regulation from that observed for cytoplasmic components in the neurons. (Supported by grants from the NIH (NS 12601), the Muscular Dystrophy Assoc., & the American Heart Association.)

227.2 FAST CHOLINERGIC AND SLOW NON-CHOLINERGIC SYNAPTIC INTERACTIONS BETWEEN ENTERIC NEURONS GROWN IN DISSOCIATED CELL CULTURE. A.L. WILLARD* & R. NISHI (SPON: S.G. MATSUMOTO). Dept. of Neurobiology, Harvard Medical School, 25 Shattuck St. Boston,MA 02115

We are studying the ability of enteric neurons from small intestines of neonatal rats to develop and differentiate in dissociated cell culture. Here we report some of their physiological properties, in particular their responses to putative transmitters and their ability to form synapses with each other. Culture conditions are described in the preceding abstract (Nishi & Willard, 1982). Sensitivity to putative transmitters was tested by using a pressure ejection system (Choi & Fischbach, 1981, J. Neurophys.45: 605) to apply "puffs" of solutions of test compounds while recording the responses of neurons with intracellular microelectrodes. Synaptic transmission was studied by impaling pairs of neurons and stimulating one while looking for responses in the other. The effects of antagonists on responses to puffs of test compounds or on synaptic potentials were examined by adding them to the perfusion medium as described by O'Lague et al(1978, Dev. Biol. 67:384.

After 3 weeks and longer, spontaneous fast epsps were observed in many cultures and 79/188 neurons tested in 20 cultures from 6 different platings caused fast epsps in one or more nearby neurons. The fast epsps could be blocked with nicotinic but not muscarinic antagonists and could be mimicked by brief puffs of ACh. The neurons often formed synaptic networks such that eliciting an action potential in one neuron caused both mono- and polysynaptic epsps in a neighbor. Six of 28 cholinergic drivers stimulated at high frequency (5-20 Hz) for 1-10 sec elicited slow depolarizations (3-15 mV) in the same neurons in which they also elicited fast epsps. The slow potentials lasted 30-200 sec and were not blocked by a combination of 500 uM hexamethonium and 10 uM atropine, suggesting that they were not caused by release of ACh.

Neurons in our cultures can be stained with antisera to 3 substances known to excite adult guinea pig myenteric neurons in vivo (North, 1982, Neurosci.7:315)--serotonin (5-HT), Substance P (SP) and Vasoactive Intestinal Peptide (VIP) (preceding abstract). Brief puffs (0.5-2 sec) of each of these compounds caused the frequency of spontaneous fast epsps to increase for 30-300 sec (5-HT, 10 uM; SP, 100 nM; VIP, 10 nM). In addition, brief puffs at these concentrations also caused long (15-200 sec) depolarizations and/or increased excitability of neurons in these cultures (47/93 for 5-HT; 20/26 for SP; 9/9 for VIP). Experiments are in progress to examine identified cholinergic drivers for their ability to be stained by antisera to these substances.

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227.3 RAT ENTERIC NEURONS IN CULTURES OF DISSOCIATED MYENTERIC AND SUBMUCOUS PLEXUS: GROWTH AND IMMUNOCYTOCHEMICAL PROPERTIES. R. NISHI & A.L. WILLARD* Dept. of Neurobiology, Harvard Medical School, 25 Shattuck St. Boston, MA 02115

The neurons of the enteric nervous system have a wide spectrum of electrophysiological, biochemical, ultrastructural and morphological properties. We have developed techniques for long-term maintainance of neurons of dissociated enteric plexuses in cell culture with the aim of studying and correlating these various properties in individual enteric neurons.

The longitudinal and circular smooth muscles with adherent plexuses (myenteric and submucous) of the first 6-8 cm of the small intestine of newborn rats are removed with fine forceps. The strips of tissue are dissociated by a combination of enzymatic digestion and mechanical disruption. Many non-neuronal cells are removed by preplating the cell suspension. Proliferation of remaining non-neuronal cells is prevented by incubating the cultures for the first 3 days in 10 uM FUdr and Ara-C. Standard growth medium consists of Eagles MEM supplemented with 5% adult rat serum. Survival was not enhanced by further supplementation with NGF, chick embryo extract, neonatal rat brain extract, heart or gut cell conditioned medium or elevated K^+ (25 mM). Elevated K^+ did enhance growth of the neurons and is therefore routinely added to growth medium. Neurons are typically maintained for 4-8 weeks in these cell cultures.

We have begun to characterize the properties of neurons at 3-6 weeks after plating. A variety of sizes (5-25 um) and shapes of cell bodies are seen. A histochemical procedure for visualizing intracellular AChE after irreversibly inhibiting extracellular AChE with phospholine iodide (Wallace, 1981, Brain Res. 219:190) labelled about 40% of neurons. Antisera directed against somatostatin, Substance P, serotonin(5-HT), VIP, and enkephalin labelled discrete subpopulations of neurons in the cultures. The largest proportion (30-40%) was labelled by anti-VIP. The smallest (5%) was labelled by anti-5-HT. Intermediate numbers (10-20%) were labelled by the other 3 antisera. The relative proportions of neurons labelled by these antisera are comparable to the relative proportions reported to be labelled in adult rat small intestines in vivo (Schultzberg et al, 1980. Neurosci. 5:689). This suggests that we are recovering a representative sample of all the neurons found in the rat myenteric and submucous plexuses. The accompanying abstract (Willard & Nishi, 1982) describes electrophysiological and pharmacological studies of enteric neurons grown in these cultures. Supported by postdoctoral fellowships from NIH and MDA to RN, by a postdoctoral fellowship from MDA to ALW, and by NIH grant NS 11576 to David D. Potter.

227.4 CAFFEINE ANTAGONIZES THE ADENOSINE-CAUSED DECREASE IN THE CA^{2+} COMPONENT OF ACTION POTENTIALS FROM RAT SENSORY NEURONS IN CULTURE. S. George Oakes* and Robert S. Pozos (SPON: D. J. Forbes). Dept. of Physiol., Sch. of Med., Univ. of Minn.-Duluth, Duluth, MN 55812.

The stimulant effects of caffeine have long been attributed to an inhibition of phosphodiesterase with a resultant accumulation of cAMP. However, because of the high concentrations of caffeine necessary to cause this inhibition, it has been suggested that methylxanthines may exert their action by blocking the receptors for adenosine (Daly, J. W., Bruns, R. F., and Snyder, S. H., Life Science 28: 2083-2097, 1981). Adenosine has depressant effects behaviorally and has been shown to inhibit the spontaneous firing of central neurons (Phillis, J. W. et al., Can. J. Physiol. Pharmacol. 57: 1289-1312, 1979). It has been suggested the depression in activity results from an inhibition of neurotransmitter release. Two-week-old dorsal root ganglia neurons with an increased Ca^{2+} component in their action potentials were utilized as a presynaptic model in this study to determine if 1) adenosine alters ionic conductances and if 2) caffeine antagonizes the adenosine effect.

Neurons were bathed in a salt solution containing 5.4 mM Ca^{2+} and 3.0 mM Ba^{2+}. Action potentials ranged from 5-300 ms in duration and the spike width was directly related to Ca^{2+} conductance. Neurons were impaled with fine-tipped micropipettes (4 M KOAc, 30-80 MΩ) and the action potentials were recorded before, during, and after drug application. The agent of interest was applied extracellularly via micropressure through microelectrodes (2-5 μm) positioned 100-200 μm away. Application of adenosine 10^{-4} M produced a marked decrease in duration without altering the resting membrane potential, spike amplitude, or maximum rate of rise. It has been calculated that adenosine 10^{-4} M is the concentration present during electrical stimulation of brain slices (Pull, I. and McIlwain, H., Biochem. J. 130: 975-981, 1972). Exposure to caffeine 10^{-4} M did not alter the action potential. However, when adenosine 10^{-4} M and caffeine 10^{-4} M were applied simultaneously, the alterations previously observed with adenosine were not seen.

These data show that adenosine causes a decrease in Ca^{2+} conductance. Decreased Ca^{2+} current at the presynaptic terminal could account for the behavioral depressant effects of adenosine. Caffeine at low concentrations may produce its stimulatory effects by blocking the action of adenosine.

227.5 SYNAPTIC PHYSIOLOGY OF DISSOCIATED HIPPOCAMPAL CULTURES. Steven M. Rothman. Division of Pediatric Neurology, Washington University School of Medicine, St. Louis, MO 63110.

There is now abundant data suggesting that glutamate (GLU) and/or aspartate (ASP) are the neurotransmitters released by excitatory neurons in the hippocampus and that GABA is the neurotransmitter used by inhibitory neurons in hippocampus. More direct evidence supporting a transmitter role for these substances in hippocampus now comes from experiments with dissociated hippocampal cultures prepared from fetal rats. This in vitro preparation allows known concentrations of transmitters and antagonists to be applied to single neurons while monitoring membrane potential. In addition, when pairs of neurons within a few hundred microns of each other are simultaneously impaled for intracellular recording, as many as 60% are found to be synaptically connected. This makes it possible to determine the effects of blocking agents on postsynaptic potentials.

In one series of experiments, bicuculline (10^{-4}M) was applied by microperfusion near pairs of neurons in which intracellular stimulation of one cell reliably produced an IPSP in the other. IPSP's were all (n=9) markedly reduced or eliminated by bicuculline and returned a few seconds after the bicuculline perfusion was stopped. No depolarizing PSP's (n=11) were affected by bicuculline. The same bicuculline concentration markedly reduced the response to directly applied GABA (10^{-4}M) but did not affect glycine (10^{-4}M) responses. As these cells never respond to norepinephrine or serotonin, the other putative inhibitory transmitters in intact hippocampus, it seems likely that GABA is used as the inhibitory transmitter in this system.

GLU (10^{-4}M) and less reliably ASP (10^{-4}M) depolarize cultured hippocampal neurons. Both GLU and ASP responses are markedly decreased by cis-2,3-piperidine dicarboxylic acid (PDA, 10^{-2}M), a recently synthesized blocker of N-methyl-D-aspartate, kainate, and quisqualate receptors. Another more selective N-methyl-D-aspartate receptor blocker, D-α-aminoadipate (ADIP, 10^{-3}M), only decreases ASP responses. PDA (10^{-2}M) does not diminish GABA responses. Of 13 pairs of neurons in which stimulation of one cell reliably produced an EPSP in the other, PDA (10^{-2}M) applied by microperfusion decreased the response in 8. In 2 pairs, where PDA and ADIP were applied sequentially, only PDA decreased the EPSP.

These results provide good evidence that GLU (and in some cases possibly ASP) is an excitatory transmitter in cultured hippocampal neurons. In addition, they suggest that selective antagonists of excitatory amino acids should help identify neurotransmitters in other systems.

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227.6 OLFACTORY BULB NEURONS IN DISSOCIATED CELL CULTURE: ACTIONS OF POSSIBLE TRANSMITTERS. Matthew P. Frosch and Marc A. Dichter. Dept. of Neuroscience, Children's Hosp. Med. Ctr., Boston, MA 02115.

Cells from 15 day fetal rat olfactory bulbs were grown in dissociated cell culture. A single cell suspension was prepared using trypsin and cells were plated on coverslips coated with collagen and poly-L-lysine. Within 24-48 hours after plating neuronal morphologies were observable; neuronal morphologies included dendritic branching patterns of both bipolar and multipolar trees, with cell body diameters from 5 to 30 μm.

In physiology experiments performed at 4 weeks, olfactory bulb cells showed a mean resting potential of 55.4 mV and a mean input resistance 73.5 MΩ (n=20). Most cells showed prominent spontaneous activity with a preponderence of EPSP's and action potentials, although IPSP's were observed. In all cells, current injection generated an overshooting action potential with a hyperpolarizing afterpotential. Pharmacologic sensitivies of olfactory bulb cells were tested with microperfusion. All cells tested were sensitive to 50 μM GABA (n=11) responding by shunting their input resistance. Cells were markedly more variable in response to glycine (100 μM), with some cells unaffected and others showing decreases in resistance. Application of glutamate caused a depolarization and an increase in both EPSP's and IPSP's at 25 and 50 μM but not at 10 μM. The presence of GABAnergic neurons in the culture was determined by 3H-GABA uptake autoradiography. Cultures at two weeks of age were exposed to 28.8 nM 3H-GABA (1 μCi/ml) for 15 min. and treated for autoradiography. After one week of exposure 20-25% of cells were positive. These included all morphologies and sizes with a tendency towards smaller neurons. 3H-GABA uptake positive cells tended to occur in groups.

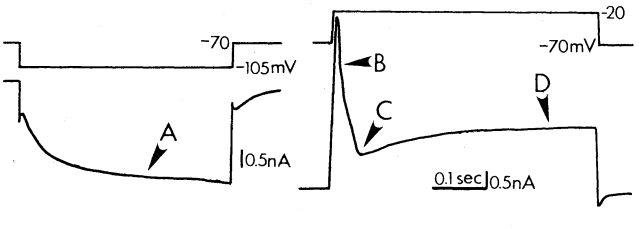
Because carnosine has been suggested as a possible transmitter for the primary olfactory neuron we examined the action of carnosine on cultured olfactory bulb neurons. Application of 100 μM carnosine caused no change in resting potential, in the input resistance, levels of spontaneous activity, nor did it potentiate the action of 25 μM glutamate. The failure of carnosine to evoke changes in membrane parameters and in levels of spontaneous activity argues against its possible role as the excitatory transmitter of the primary olfactory neurons. It is possible, however, that the cultures did not contain mitral cells (the natural target of the primary sensory fibers) or that the presence of innervation by olfactory nerves is required for the development of sensitivity.

Supported by NIH grants NS15362, 1T32GM07753, and the CHMC Mental Retardation Core Grant HD06276.

227.7 VOLTAGE CLAMP ANALYSIS OF CULTURED RAT HIPPOCAMPAL NEURONS. Menahem Segal* and Jeffery L. Barker. Lab. of Dev. Neurobiology, NICHHD and Lab. of Neurophysiol., NINCDS, NIH, Bethesda, MD 20205.

The two-electrode voltage clamp technique was used to analyze the conductance mechanisms resident in cultured rat hippocampal neurons. The cells were dissociated from 17 to 19 day old rat embryos and grown in culture for 4 to 6 weeks. Pyramidal and multi-polar cells (10-20 μm dia.) were studied at room temperature using phase contrast optics. Stable recordings with two KCl-filled microelectrodes could be maintained for up to two hours. The cells exhibited resting potentials of -35 to -65mV, input resistances of 10 to 40MΩ and action potentials of 50 to 80mV, as well as spontaneous synaptic potentials. Electrically excitable membrane conductances were examined by clamping the cells to -70mV and stepping the membrane over the range -120 to +10mV. Hyperpolarizing commands elicited inward-going, time-dependent, Cs^+-sensitive current responses (A). Depolarizing commands evoked 1) fast inward currents eliminated by TTX, 2) rapidly rising outward currents which fully inactivated in 50 msec and were attenuated by 10mM 4-aminopyridine (B), 3) slow inward currents blocked by Co^{2+} (C), 4) slowly rising outward currents depressed by 25mM TEA, and 5) slowly rising outward currents resistant to TEA and blocked by Co^{2+} (D).

All hippocampal neurons responded to application of GABA with an increase in membrane conductance to Cl^- ions over the entire range of potentials studied. Conductance increases were routinely greater at +10mV than at -90mV. Analysis of the fluctuations in membrane current which occurred during the responses revealed spectra characteristic of two-state (open-closed) ion channel activity. Estimates of the channel properties activated by GABA revealed conductances in the range 15-30 pS and lifetimes in the range 10-20 msec. Thus, cultured hippocampal neurons exhibit electrically and chemically excitable membrane conductances whose mechanisms are similar to those described in other excitable membranes. Quantitative analysis of these functions should improve our understanding of their physiological roles in central mammalian neurons.



227.8 SUSTAINED HYPEREXCITABILITY FOLLOWING ELECTRIC STIMULATION OF ORGANOTYPIC HIPPOCAMPAL EXPLANTS. J. Fowler and S.M. Crain Dept. of Neuroscience, Albert Einstein Coll. Med., Bronx, N.Y.

Previous studies have demonstrated that long-lasting seizure-like activity can be generated in organotypic explants of fetal mouse hippocampus during maturation in culture (Crain and Bornstein, Br. Res. 68 '74). Spontaneous and evoked discharges in these explants often showed remarkable similarities to epileptiform activities recorded in situ. Intracellular recording from hippocampal explants (2-8 wks in vitro) revealed long-lasting EPSPs and IPSPs, as well as paroxysmal activity (Zipser et al, Br. Res. 60 '73). In the present study transverse slices (ca. 0.5 mm thick) of the hippocampus were explanted from late fetal or neonatal mice onto collagen-coated coverslips (culture technique in C. & B. '74). Extracellular field potentials were recorded from these explants after 1.5-2 wks in culture. Recording electrodes (3-5u tips) were placed in s. radiatum or s. oriens areas of CA-1 or CA-3. Stimulation was applied via 10u electrodes placed in the dentate area. Stimulation parameters were varied within and between experiments but in general consisted of monophasic pulses (0.5-1 msec; 5-20 uamps). Spontaneous slow-wave discharge patterns were mapped at specific sites prior to application of electric stimuli. These discharges ranged from 300 uV to more than 1 mV in amplitude and from 60 msec to 1-2 seconds in duration, persisting in many cases for the duration of the experiment (up to 30 hrs). Sustained hyperexcitability was elicited following three stimulation paradigms. 1) When the amplitude of the primary component (50-100 msec) of spontaneous slow-wave discharges was measured before stimulation and then after 4 trains of pulses (1/sec for 2-4 sec) at 5 minute intervals, an amplitude increase of 50% was observed in some trials. 2) When similar brief trains of stimuli (constant strength) were applied at 2-10 min intervals the amplitude of the primary component of the evoked slow wave response increased by as much as 40% and persisted for as long as 5 mins. 3) When stimulus bursts (100/sec for 1 sec) were applied at 2-10 min intervals spontaneous discharges were often elicited after 2 or more stimulus trains; in other cases the frequency of discharges progressively increased during the 0.5-1 min test interval. In all three series the form of the evoked slow-wave discharge was within the range found with spontaneous discharges at the same sites. These results indicate that electric stimulation can induce sustained hyperexcitability changes in organotypic explants of hippocampus and will complement analyses carried out in vivo and in other types of in vitro models. [Supported by NIH grant (NS-14990) to S.M.C. & predoctoral training grant (Sue Golding Graduate Division of Albert Einstein Coll. Med.) to J.F. Tissue culture facilities (NIH grant NS 11920) were kindly provided by Dr. Murray Bornstein.]

227.9 Ca^{2+} DEPENDENT, CAFFEINE SENSITIVE POTENTIAL FLUCTUATIONS IN CULTURED DORSAL ROOT GANGLION CELLS OF THE MOUSE. David A. Mathers* and Jeffery L. Barker (Sponsor: Henry G. Wagner) Laboratory of Neurophysiology, NINCDS, National Institutes of Health, Bethesda, Maryland 20205.

Using intracellular recording techniques, we have studied the spontaneous voltage and current fluctuations recently reported at the membrane of cultured mouse dorsal root ganglion (DRG) cells (Mathers, D.A. and Barker, J.L., Brain Res., 211: 451, 1981). At the resting potential, 101/177 DRG neurons tested showed spontaneous subthreshold potentials which took two forms: (a) high frequency voltage fluctuations a few millivolts in peak-to-peak amplitude and (b) small discrete hyperpolarizations of amplitude 1.9±0.4 mV, time to peak 7.2±1.7 msec and 1/2 decay time 9.9±2.8 msec (mean ± S.D. for >100 events measured in 5 cells at 23°C). Neurons displaying either type of events were designated as active DRG cells.

When 5 active cells showing the discrete type of voltage event were voltage-clamped at the resting potential, discrete outward currents of mean amplitude 78±29.7 pA and duration 23±8.8 msec were seen. The amplitude distribution of membrane current fluctuations recorded from active DRG cells was approximately Gaussian at clamp potentials more negative than -65 mV, at which spontaneous outward currents were suppressed. On depolarization, the distribution became increasingly skewed in the positive direction. These data suggest that active DRG cells possess an outward (positive going) component of membrane current which is activated discontinuously on depolarization.

The spontaneous voltage fluctuations of active DRG neurons were abolished in Ca^{2+} free medium and of the divalent metal cations Sr^{2+}, Mg^{2+}, Ba^{2+}, Co^{2+} and Mn^{2+}, only Sr^{2+} could substitute for Ca^{2+} in the maintenance of this activity. 1-10 mM tetraethylammonium blocked the spontaneous potentials while 10 mM caffeine increased the frequency of these events. The spontaneous voltage fluctuations were not dependent on the presence of spinal cord neurons in the culture plate, and were also seen in cultured DRG cells obtained from adult mice. The potentials seen in active DRG cells closely resemble events recently reported in cultured bullfrog sympathetic ganglion neurons (Adams, P.R., Brown, D.A., Constanti, A. and Adams, C.E.Y., Biophys. J., 37: 308a, 1982). Both forms of subthreshold activity may share a common origin in the activation of a Ca^{2+} dependent K^+ conductance by Ca^{2+} released from intracellular stores.

227.10 RECORDING OF SPONTANEOUS ACTIVITY FROM MONOLAYERS OF MOUSE SPINAL NEURONS GROWING ON PHOTOETCHED MULTIMICROELECTRODE PLATES. G. W. Gross and J. H. Lucas*. Department of Biology, The Texas Woman's University, Denton, TX 76204.

Vigorous spontaneous activity has been obtained from monolayers of dissociated mouse spinal neurons seeded onto surfaces containing a fixed matrix of 36 photoetched gold conductors. The pickup of extracellular signals was achieved with shallow craters 3 µm deep and 10 to 15 µm in diameter which were shot into the insulation layer with UV laser pulses. Electrode impedances ranged from 3 to 6 megohm; shunt impedances were above 35 megohm. Activity was recorded as early as 6 days postseeding, but most data has been gathered 2 to 4 weeks postseeding. Experiments were performed with average cell densities of 300 neurons/mm^2 which allowed identification of all cells and major processes adhered to the polysiloxane insulation material. We have recorded activity for several days with maximum signal-to-noise ratios of 30:1 and maximum frequencies of 300 Hz from neurons only 15 µm diameter. The largest signals were obtained from neurons residing in or covering the recording crater. Neurons more than 30 µm from a crater could not be detected. Integrated activity from individual electrodes over a period of several days usually showed patterns of random bursting interrupted by periods of intense steady firing. These patterns showed remarkable stability with time except for gradual increases in signal-to-noise ratios and lengthening of steady firing periods. Activity has also been obtained from axon bundles floating above recording craters. These results represent a critical test of the applicability of fixed matrix, photoetched multimicroelectrode surfaces to the problem of recording long-term, simultaneous activity from many neurons growing in a two-dimensional pattern in culture chambers. Technical and neurobiological difficulties have heretofore frustrated all efforts to monitor activity from dispersed monolayers of visible CNS neurons using similar approaches. Network tailoring via laser cell surgery, continuous optical monitoring of network development, pharmacological probing, and systematic stimulation are now realistic goals that can be addressed in the near future.

Supported by NIH Grant NS15167

227.11 CHARACTERIZATION OF FETAL RAT BRAIN CELLS GROWN IN A DEFINED MEDIUM. Z. Ahmed*, P. Walker*, and R.E. Fellows. Department of Physiology and Biophysics, The University of Iowa, Iowa City IA 52242.

A procedure has been developed for the maintenance and growth of dissociated cells from cerebrum, cerebellum, and brain stem of the 20-day gestational fetal rat for 2-6 weeks in primary culture. A unique feature of this method is the complete elimination of a requirement for serum, either for plating or during extended culture (Ahmed, Walker and Fellows, Fed Proc, 1982). This methodology was developed to eliminate uncontrolled variables introduced into conventional culture systems by biologically active components of serum. Cells prepared by this procedure attach to a polylysine substratum in less than 20 minutes and extend processes within hours. Measurement of DNA and protein of these cells indicates the presence of a stable population of viable cells with limited cell proliferation. Indirect immunofluorescent staining of two-week-old cultures with tetanus toxin as a neuron surface-specific marker, horse-anti-tetanus toxoid serum, and fluorescein-labeled goat anti-horse-IgG demonstrates a preponderance of cells with processes which bind tetanus toxin. These cells also have gross morphological properties consistent with their classification as neurons. The majority of cells in cultures of combined cerebellum and brain stem stain with glyoxyalic acid, which exhibits specific fluorescence upon reaction with catecholamines. A smaller proportion of cells from cerebrum demonstrate fluorescence after exposure to this reagent. Microelectrode recording from morphologically identifiable cells in these cultures has shown their ability to generate action potentials. After 4-5 days in culture, action potentials rarely show overshoot and have durations more than two times longer than mature neurons. By 10 days in culture, these characteristics have disappeared and the size and shape of the action potential resemble those of mature mammalian neurons in situ. This culture system provides an excellent in vitro model for studies of the developmental and regulatory biology of mammalian brain at the cellular level. (Supported by NIH Grants HD13154 and HL24402.)

227.12 EFFECT OF ANGIOTENSIN II ON NOREPINEPHRINE LEVELS IN PRIMARY CULTURES OF RAT BRAIN. Colin Sumners, M. Ian Phillips and Mohan K. Raizada, Department of Physiology, University of Florida College of Medicine, Gainesville, FL. 32610.

Angiotensin II (AII) is known to interact with norepinephrine (NE) in the periphery and in the brain. The biological effects of AII on the brain are in part mediated by the catecholamines (CA) although the mechanisms of this interaction is not fully understood. Neuron-enriched primary cultures of one-day-old rat brains have been utilized in this investigation to study the interactions between AII and NE without the confounding variables of the in vivo condition. Our previous studies have demonstrated that these cultures contain AII receptors and CA on selected populations of neurons. Brains from one-day-old rats were dissociated with trypsin, cells were plated on polylysine-coated culture dishes and grown in DMEM containing 10% horseserum and 5% FBS. Neuron enrichment of these cultures was achieved by treatment with cytosine arabinoside. Three-week-old brain cell cultures which contained up to 80% neuron were used for the experiments. NE content in the media and cells was determined by radioenzymatic method after incubation of these cultures with AII for 24 hr. The results are shown in the Table.

Cells	Control	AII (µg/ml) 0.75	7.5	15
NE (cells) pg/mg protein	1102±57(7)	808±30(6)	1365±104(6)	1516±90(6)
NE (media) pg/ml	240±29(6)	263±19(6)	362±40(6)	399±31(6)

Lower doses of AII (0.75 µg/ml) caused a 27% decrease in the neuronal norepinephrine levels. Doses of 7.5 and 15 µg/ml AII caused a 24, 38% increase in cell NE and a 51, 60% increase in the media NE, respectively. Saralasin, the AII receptor antagonist, completely inhibited the NE stimulation by AII. This observation suggests that the effect of AII may be mediated by its cell surface receptors on the neurons. The results implied that at the low dose of AII there may be a release of NE whereas at high dose there may be both release and increased synthesis of NE. These results demonstrate that AII directly influences NE levels of neurons and offers an in vitro system to study whether the changes are in the synthesis, release, reuptake or metabolism.

227.13 DUAL ELECTROPHYSIOLOGICAL EFFECTS OF PHENYTOIN ON NEURAL TISSUE CULTURES. F.J. Seil and A.L. Leiman*. Neurology Research, V.A. Medical Center and Department of Neurology, Oregon Health Sciences University, Portland, OR and Department of Psychology, University of California, Berkeley, CA.

The electrophysiological effects of direct application of phenytoin to CNS tissue were assessed in cerebellar and cerebral neocortical explants. The cultures were exposed to concentrations of phenytoin ranging from 2 to 22.5 µg per ml of buffered balanced salt solution while extracellular electrical activity was recorded. Dual electrophysiological effects were noted in both culture systems. In cerebellar explants, an initial potentiation effect consisted of a marked enhancement of spontaneous activity of Purkinje cells. The duration of this effect was dose dependent, and at low doses only excitatory effects were evident. This effect was also noted at several stages of in vitro development. At higher doses, the potentiation of cerebellar cortical discharges was followed by persistent (and reversible) depression. Marked sensitivity to phenytoin was evident in some cerebellar cultures. A similar dual effect of phenytoin application was also evident in stimulus elicited responses of cerebral neocortex explants. A marked enhancement of the amplitudes of these responses lasted from several minutes to as long as 1/2 hour. Increased oscillatory activity with prolonged bursting of spikes was also recorded. The later effects of depression were evident in all phases of cerebral neocortical evoked responses.

The actions of phenytoin in neural tissue cultures revealed a complex temporal variation that included a phase of heightened excitability followed by more persistent depression. This was seen in cultures from different CNS regions, although a special sensitivity was evident in cerebellar explants. The initial enhanced excitability produced at all dose levels of phenytoin may have particular significance for some clinical uses of this drug as an anticonvulsant.

Supported by the Veterans Administration.

227.14 ELECTRICALLY EXCITABLE HUMAN MACROPHAGES IN SHORT TERM CULTURE. F.V. McCann, J.J. Cole and P.M. Guyre*, Department of Physiology, Dartmouth Medical School, Hanover, NH 03755.

Overshooting action potentials can be elicited by current injection into peripheral blood monocyte macrophages (MΦ) isolated from normal human donors. Of the 13 cells reported in this study with action potentials, resting potentials achieved -72 mV. Subthreshold currents injected into each cell elicited a graded potential. The steady state input resistance was 30-40 MΩ and the time constant was 2-5 msec. The diameter of the attached cells was 30-50 µm, and for a typical cell 10 µm thick the specific membrane resistivity was 1,600 Ω cm^2. The membrane often ruffled and produced conical processes. While graded potentials could be produced with small current injections, the action potential always required 0.5 nAmp or more when delivered as a rectangular pulse. Threshold was in all cases more positive than -5 mV. The action potential was always overshooting, tracing a slower upstroke and a faster down stroke. The time from current-on to the most negative post-spike hyperpolarization was 50 msec or less. Repetitive spiking of 20-40 Hz was positively related to stimulus intensity. Functional maturation began when the monocyte adhered to the substrate, resulting in a large resting potential and spiking capability by 6-8 days in culture.

Besides its intrinsic value as a new class of spiking mesodermal cell, the functional maturation of the macrophage spike in culture closely parallels the maturation of Fc receptors, and may be a membrane electrical correlate for recognition or other cell surface functions. (Supported by NIH, BRSG #05392.)

228 SYMPOSIUM. NEUROBIOLOGY OF ANXIETY. H. Lal, Co-Chairperson, Texas College of Osteopathic Medicine; E. Costa, Co-Chairperson, NIMH; W.E. Bunney, Jr., Univ. California; S. Fielding, Hoechst-Roussel Pharmaceuticals; A. Guidotti, NIMH; C. Braestrup*, Sct. Hans Hospital; D.E. Redmond, Jr., Yale Univ.

Normal anxiety is a biological state that provides adaptive advantages in serving as the basis for caution and the emotional patterns of the intellectual comparison between potential cost and benefit of any action. It thus has an important role in the regulation of motivated behavior. Maladaptive anxiety interferes with body functions that may result in psychosomatic illnesses. Although the importance of anxiety in health and disease is recognized, the neuro-biological basis of its manifestation is not known. The symposium will review diagnosis, manifestations, and neuro-biological mechanisms of anxiety and its disorders. It will compare paradigms of animal behavior that use either go no-go conflict or suppression of punished responses, with those which use chemically-induced behavioral state to mimic anxiety. Value of discriminative stimuli produced by anxiogenic drugs such as pentylenetetrazol, yohimbine or B-carbolines, will be assessed with respect to providing an animal model of anxiety. Experimental activation or electrolytic lesions of the nucleus locus coeruleus alter anxiety and fear-associated behaviors in monkeys. Pharmacological experiments will be presented to support the hypothesis that this central noradrenergic nucleus is a site of "alarm" system. GABA-benzodiazepine-ionophore complex has been proposed to be the site of action of anxiogenic as well as anxiolytic actions of drugs. Isolation and function of a protein, GABA modulin, will be described with respect to effects on benzodiazepine-receptor interaction and behavior. B-carbolines and related compounds will be reviewed with respect to their anxiogenic actions and affinity to non-GABA stimulated conformation of benzodiazepine receptors.

229 SYMPOSIUM. FUNCTIONAL CORRELATES OF BRAIN TRANSPLANTATION. B.J. Hoffer*, Univ. Colorado Health Sciences Center (Chairman); L. Olson*, Karolinska Institute; D. Gash, Univ. of Rochester; D. Krieger, Mt. Sinai Medical Center; W. Freed, NIMH, St. Elizabeth's Hospital.

This symposium will focus on functional correlates of brain transplantation, utilizing anatomical, electrophysiological, endocrinological and behavioral approaches. The in oculo transplant model will be presented first, with histochemical and electrophysiological evidence for transmitter-mediated functional interactions between multiple grafts. Recent studies on growth regulation in cerebral cortical grafts will also be presented. Data from transplants of vasopressin-containing neurons into the hypothalamus of vasopressin-deficient Brattleboro rats, which manifest diabetes insipidus, will be described next. This data suggests that such grafts survive and develop cytoarchitectural features of normal hypothalamus. Axons from vasopressin-containing neurons in the graft invade the host median eminence and can partially reverse the endocrine disturbance, restoring urine concentrating ability in these animals. In a similar vein, recent experiments will be presented on gonadotropin-releasing hormone (GnRH)-deficient male mice. Transplants of preoptic area into the third ventricle of deficient mice manifest GnRH-positive neurons with fibers passing to capillaries in the host median eminence. As compared to untreated animals, gonadotropin levels were increased and evidence for spermatogenesis was found. Finally, studies on transplants of substantia nigra to dopamine (DA)-denervated rat caudate will be described. Histochemical and electrophysiological evidence for organotypic maturation of DA neurons in the transplant will be presented, as well as behavioral evidence for a functional DA input to the host animal's striatum. Grafts of adult chromaffin cells from the adrenal medulla also survive well in the lateral ventricle and induce behavioral effects similar to those seen with nigral grafts. The participants will conclude with a discussion of the future implications of brain transplantation for both basic and clinical neuroscience.

230.1 INHIBITION OF PRIMATE SPINOTHALAMIC NEURONS BY STIMULATION OF THE THORACIC VAGUS. W. Steve Ammons, Robert W. Blair and Robert D. Foreman. Dept. of Physiology & Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

Previous studies in our laboratory demonstrated that activation of cardiopulmonary sympathetic afferent fibers by electrical stimulation (Blair et al, J. Neurophysiol. 46:797, 1981), intracardiac injections of bradykinin (Blair et al, Circ. Res., in press, 1982) or coronary artery occlusion (Ammons et al, Fed. Proc. 41:1517, 1982) caused increased discharge of spinothalamic tract neurons of the primate. Although such noxious cardiac events also activate vagal afferent fibers, the role of these fibers in altering spinothalamic neuronal activity is unknown. Thus we decided to determine the responsiveness of spinothalamic neurons to electrical stimulation of the thoracic vagus nerve. All experiments were performed on monkeys (Macaca fascicularis) tranquilized with Ketamine and anesthetized with α-chloralose. The T_1 to T_5 segments of the spinal cord were searched for antidromic responses to electrical stimulation of the contralateral ventral posterior lateral nucleus of the thalamus. Each cell studied was thus identified as a cell of origin of the spinothalamic tract. In addition all cells studied were responsive to manipulation of their somatic fields and were activated by electrical stimulation of the caudal ansa subclavia. 37 spinothalamic neurons receiving viscerosomatic convergent inputs were studied for responses to stimulation of the left thoracic vagus (10-30V, 2 msec, 20 hz) for 5-10 sec. 32 cells exhibited background activity and of these 19 were inhibited during vagal stimulation from a control rate of 17 ± 3 to 5 ± 2 spikes per second. 10 cells were unresponsive (14 ± 3 to 15 ± 3 spikes per sec), 2 were excited, and 1 was initially excited and then inhibited. Vagal inhibition of sympathetic afferent input was assessed with the conditioning testing technique. For 4 of 8 cells such condition-testing curves indicated a prolonged period of inhibition due to vagal stimulation raising the possibility of involvement of a presynaptic mechanism. Vagal effects on somatic responses were determined for 12 cells. Pinch of the skin or muscle overlying the somatic field elevated background activity from 6 ± 2 to 33 ± 5 spikes per second. At the peak of the response left thoracic vagal stimulation was imposed resulting in reduction of the discharge rate to 13 ± 2 impulses per second. The ability of the vagus to inhibit cell activity did not appear to be related to the type of sympathetic fiber input, the somatic field type, or cell location. Presumably these effects involve activation of vagal afferent fibers with information traversing the brainstem and descending spinal pathways. Supported by National Heart, Lung and Blood Institute Grants HL22732, HL07430 and HL00557.

230.2 EFFECTS OF STIMULATING THE NUCLEUS RAPHE MAGNUS ONTO SPINORETICULAR NEURONS OF THE T_2-T_4 SEGMENTS IN THE SPINAL CORD OF THE CAT. C. Dale Chapman,* W. Steve Ammons and Robert D. Foreman. Univ. of Oklahoma Health Sciences Center. Dept. of Physiology and Biophysics, Oklahoma City, OK 73190.

Electrical stimulation of the nucleus Raphe Magnus (RM) produces analgesia and inhibits noxious input to dorsal horn neurons of the spinal cord. The objective of this study was to determine the effects of RM stimulation on somatic and visceral inputs onto spinoreticular (SR) tract neurons. Our laboratory showed that the SR tract transmits primarily noxious and secondarily innocuous information. We have also demonstrated that SR neurons receive convergent input from somatic and visceral (cardiopulmonary) receptors.

In cats anesthetized with α-chloralose the RM was electrically stimulated with a bipolar electrode at a current intensity of 50-700 μA. The neurons of the SR tract were antidromically activated by stimulation of the medullary reticular formation. These stimulating electrodes were placed bilaterally at approximately P10 and 1.5 to 2 mm lateral from midline. The activity of SR neurons in the upper thoracic spinal cord (T_2-T_4) was recorded before and during RM stimulation. The left sympathetic afferent fibers were stimulated with bipolar hook electrodes placed on the ansa subclavia to demonstrate visceral input. All of the SR neurons tested were shown to have both somatic and visceral inputs. Condition-Test curves were built by testing the response of the SR neurons to stimulation of sympathetic afferent fibers after conditioning with RM stimulation. The RM was also stimulated during noxious or innocuous stimulation of the somatic field of the SR neurons.

Stimulation of the RM inhibited the background activity of all 17 SR neurons tested. By increasing the current to 700 μA 13 of the 17 SR neurons were completely inhibited for the duration of the stimulation. Noxious somatic input such as pinching the skin or muscle as well as innocuous input such as brushing the hair were inhibited during RM stimulation. The condition-test curve shows inhibition of visceral input with a prolonged duration.

It can be concluded that stimulation of the RM can inhibit either somatic or visceral input onto SR neurons. The inhibition is effective for information of a noxious or innocuous nature.

Supported by National Heart, Lung and Blood Institute Grants HL22732, HL07430, HL00557.

230.3 INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION BY MEDIAL PREOPTIC AND SEPTAL STIMULATION. J.D. MacKinnon & E. Carstens. Dept. of Animal Physiology, Univ. of Calif., Davis, CA 95616.

Electrical stimulation at medial brainstem sites produces analgesia which is associated with powerful descending inhibition of spinal dorsal horn neuronal responses to noxious inputs. Analgesia also results from stimulation at more anterior sites in the medial preoptic and septal areas. To investigate whether these analgesic effects might be mediated by descending inhibition of spinal nociceptive transmission, we tested whether stimulation in these areas inhibited the responses of spinal dorsal horn neurons to controlled noxious radiant skin heating.

In cats anesthetized with sodium pentobarbital and 70% N_2O, the responses of single lumbar dorsal horn units to noxious radiant heat stimuli (50°C, 10 sec) applied to glabrous hindfoot skin were recorded with tungsten microelectrodes. The heat-evoked responses of all units studied were markedly reduced during concomitant electrical stimulation (100 msec trains at 100 Hz, 3/sec, 25-400 μA) at histologically localized sites in the medial preoptic and ventromedial septal areas. Brain sites at which stimulation inhibited spinal unit heat-evoked responses were mapped by systematically varying the depth of the stimulating electrode along tracks at anterior levels +14 to +18. At each stimulation site, the magnitude of the unit heat-evoked response during brain stimulation was expressed as a % of the control response without brain stimulation, which was stable in size over repeated trials. Inhibitory sites were located in the medial preoptic area and ventromedial septum (diagonal band of Broca) up to anterior level +17. Stimulation at more anterior levels was either ineffective or produced mild facilitation of unit heat-evoked responses. The magnitude of inhibition increased with graded increases in brain stimulation intensity. For 15 units, the mean current strength at threshold for generating inhibition was 25 μA. This was lower than thresholds for inhibition from the medial hypothalamus (67 μA) and midbrain periaqueductal gray (PAG)(170 μA). The responses of dorsal horn units to a series of graded noxious heat stimuli increased linearly from threshold (40-45°C) to 52°C. When the temperature series was repeated during concomitant medial preoptic or septal stimulation in 10 units, the slope of the linear temperature-response function was significantly reduced with no change in the response threshold. This effect was similar to that produced by medial hypothalamic and midbrain PAG stimulation.

The ventromedial septal area lies at the anterior extent of a medial inhibitory system which appears to be continuous posteriorly with the medial hypothalamic periventricular gray and midbrain PAG.

230.4 INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION BY MEDIAL HYPOTHALAMIC STIMULATION. E. Carstens. Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

Electrical stimulation of the midbrain periaqueductal gray (PAG) and medullary raphe nuclei produces analgesia which is associated with powerful descending inhibition of spinal dorsal horn neuronal responses to noxious inputs. Analgesia also results from stimulation of the medial diencephalon. To investigate whether this analgesia might also be associated with descending spinal inhibition, electrophysiological methods were used to test whether the responses of dorsal horn neurons to noxious skin heating could be inhibited by medial diencephalic stimulation.

The responses of single lumbar dorsal horn units to controlled noxious radiant heat stimuli (50°C, 10 sec) applied to glabrous hindfoot skin were recorded with tungsten microelectrodes in cats anesthetized with sodium pentobarbital and N_2O. The heat-evoked response of each of more than 50 units was markedly reduced during concomitant electrical stimulation (100 msec trains at 100 Hz, 3/sec, 50-400 μA) via a bipolar electrode stereotaxically positioned at sites which were histologically localized to the periventricular gray (PVG) region of the medial hypothalamus. Inhibitory stimulation sites were systematically mapped by varying the depth of the stimulating electrode along tracks through the medial diencephalon at anterior levels +5 to +13. At each stimulation site, the magnitude of the unit's heat-evoked response during brain stimulation was expressed as a % of the unit's control response without brain stimulation. The most effective inhibitory sites were centered in the PVG bilaterally at each anterior level. Inhibition was also generated from the medial thalamus. The magnitude of inhibition increased with graded increases in PVG stimulation intensity. For 15 units, the mean current strength at threshold for generating inhibition was 67 μA. Dorsal horn unit responses to a series of graded noxious heat stimuli increased linearly from threshold (40-45°C) to 52°C. When the temperature series was repeated during concomitant PVG stimulation, the slope of the temperature-response function was reduced without a significant change in the response threshold for each of 12 units. This effect was similar to that produced by midbrain PAG stimulation. The spinal inhibitory effect of PVG stimulation was blocked by lesions of the dorsal spinal cord rostral to the recording site, indicating that inhibition was mediated via a pathway descending in the dorsal spinal white matter.

The results indicate that activation of a medial system, extending from the midbrain PAG through the diencephalic PVG into the basal forebrain, can powerfully inhibit spinal dorsal horn neurons which are involved in transmitting nociceptive information.

230.5 EFFECT OF SEROTONIN AND NOREPINEPHRINE ON RAPHE-SPINAL CELLS, Wm.S. Willcockson*, K.D. Gerhart, C.L. Cargill* and W.D. Willis. Marine Biomed. Inst., Depts. Physiol. Biophys. & Anat., Univ. Tex. Med. Br., Galveston, TX 77550.

The nucleus raphe magnus (NRM) has been implicated in the descending inhibition of pain responses. Stimulation in NRM produces analgesia, possibly by inhibiting spinal cord nociceptive neurons. Analgesia and inhibition of spinal nociceptive neurons also results from stimulation of the periaqueductal gray (PAG). It has been proposed that these actions are mediated via a synaptic link between PAG and NRM and the adjacent reticular formation (Basbaum and Fields, 1978). Serotonin (5HT) is thought to be a synaptic transmitter in this descending system. Curiously, 5HT receptor blockers are more effective in preventing PAG than NRM inhibition of primate STT cells (Yezierski et al., 1982). One explanation of this paradox would be that PAG excites raphe-spinal cells by release of 5HT. By contrast, it is thought that norepinephrine (NE) inhibits raphe-spinal cells, since phentolamine, a NE antagonist, produces analgesia when injected into NRM (Hammond et al., 1980).

To test these hypotheses, we ejected 5HT and NE by microiontophoresis into the vicinity of raphe-spinal (RST) neurons in the monkey, Macaca fascicularis. RST neurons were identified by antidromic activation following stimulation of the dorsolateral funiculi of the spinal cord at an upper lumbar level. Antidromic latencies were from 2.1 to 18.0 ms, implying axonal conduction velocities appropriate for small myelinated axons.

Drugs were applied iontophoretically from 7-barrelled glass microelectrodes. Extracellular spikes were recorded from the center barrel, which contained a low-resistance carbon fiber. Drug concentrations and pH's were as follows: glutamate, 0.2M, pH 8.5; 5HT, 0.05M, pH 5.0; quipazine, 0.05M, pH 5.0; NE, 0.1M, pH 3.9. Many RST cells had a low level of background discharges and so their activity was enhanced by pulsed release of glutamate at 10 s intervals (7 to 150 nA). The actions of 5HT, quipazine (a 5HT agonist) and NE were tested against the activity evoked by the glutamate pulses or, in some cases, against the excitation of RST cells by stimulation in the PAG.

5HT, quipazine and NE produced a current-dependent inhibition of the responses to glutamate pulses in almost all RST cells tested. No cases of excitation were found. Threshold inhibition required 25-150 nA for the different drugs. 5HT also inhibited PAG excitation of 7/10 RST cells and NE inhibited PAG excitation of 3/6 RST cells.

We conclude that both 5HT and NE inhibit raphe-spinal cells. (The work was supported by NIH grants 09743 and NS 11255 and by a grant from The Moody Foundation.)

230.6 ULTRASTRUCTURE OF SPINAL CORD TERMINATIONS OF PHYSIOLOGICALLY IDENTIFIED AXONS DESCENDING FROM MIDLINE MEDULLA AND PONS. A.R. Light and A. Kavookjian *. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

Descending projections from the midline rostral medulla and caudal pons have been implicated in the inhibition of afferent nociceptive activity at the spinal level. In order to understand the mechanisms by which such inhibition could occur, single axons descending from brainstem n. raphe magnus were recorded in the spinal cord dorsolateral funiculus and stained by iontophoresis of horseradish peroxidase through the recording micropipette. Selected labeled arborizations were reconstructed at light level and recut into ultrathin sections for examination with the electron microscope.

At least two different patterns of projections into the spinal grey matter were observed. In anesthetized cats the two patterns were not related to the evoked, ongoing activity, or conduction velocities of the fibers, but were related to the brainstem region from which the fiber was presumed to have originated. Fibers originating from the medullary raphe terminated in more ventral laminae (V-X and intermediolateral cell column (IML)). Fibers originating from caudal pontine raphe terminated more dorsally,in laminae I, outer II (IIo), V, X and IML. The boutons in laminae V-X were usually dome-shaped structures, containing clear, round or oval vesicles and many larger dense core vesicles. Synaptic contacts were made by each bouton with the synaptic density being intermediate (i.e., neither clearly symmetric nor asymmetric). Contacts were made with larger dendritic shafts, often at the exit of dendritic appendages. Other contacts were found on dendritic spine shafts.

Boutons in laminae I and IIo were similar in shape and vesicle content and densities were again of the intermediate variety. However, contacts were found on dendritic spine heads, and large proximal dendrites, and on somas. In addition, a few of the boutons were found to be presynaptic to unlabeled axon terminals in an axo-axonal arrangement.

The data suggest that the midline medulla and pons may modulate motor and reflex interneurons as well as sensory neurons, and that this modulation could be accomplished through synapses placed in potent positions on cell somas or proximal dendrites. Finally, they demonstrate the possibility of direct presynaptic influences from some descending fibers.

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230.7 DIFFERENTIAL DISTRIBUTION OF SEROTONERGIC AXONAL CONTACTS ON IDENTIFIED CAT DORSAL HORN NEURONS AND CORRELATION WITH NUCLEUS RAPHE MAGNUS STIMULATION. V. Miletic, M.J. Hoffert, M.A. Ruda, Y. Shigenaga* and R. Dubner. Neurobiology & Anesthesiology Br., NIDR, NIH, Bethesda, Maryland 20205.

Serotonergic (5HT) pathways appear to be involved in the descending modulation of dorsal horn neuronal activity. This study examined the distribution of 5HT axonal contacts on identified neurons in the superficial dorsal horn by combining the intracellular HRP method with immunocytochemistry. In some experiments the 5HT distribution was correlated with effects produced by electrical stimulation within the nucleus raphe magnus (NRM).

Neurons in the lumbar spinal cord were characterized physiologically by their responsiveness to natural and electrical stimulation of the skin. These neurons were then injected intracellularly with HRP. The spinal cord was sectioned and reacted with $CoCl_2$-intensified DAB to stain the filled cells with a blue-black chromagen. The tissue was then processed for 5HT using the PAP method to stain 5HT axons with a red-brown chromagen. Camera lucida drawings of the HRP-filled cells and adjacent 5HT immunoreactive axons were made using a 100X objective. The NRM was stimulated with a monopolar electrode, the tip of which was placed 8 mm rostral to the obex and 5 mm below the floor of the exposed fourth ventricle. Results are based on an analysis of a total of 24 nociceptive-specific (NS), wide-dynamic-range (WDR) and low-threshold mechanoreceptive (LTM) neurons located in laminae I and II. NS and WDR marginal (n = 13) and stalked cells (n = 6) received a significantly larger number of 5HT axonal contacts ($\bar{x}$ = 70) than NS and WDR IIa islet cells (n = 3, $\bar{x}$ = 25 contacts). LTM lamina II cells (n = 2) received less than 10 axonal contacts. Most of the axonal contacts on nociceptive marginal and stalked cells occurred within the proximal 200 µm of the dendritic tree. In contrast, the far fewer contacts on IIa islet cells occurred more frequently on distal dendrites. Irrespective of the type of cell, however, the 5HT axonal contacts occurred predominantly on dendritic shafts rather than spines. With NRM stimulation, the responsiveness of marginal and stalked cells to noxious stimulation was markedly attenuated. In some cases the innocuous input to WDR units was also affected, but to a lesser degree. NRM stimulation did not affect the responsiveness of nociceptive IIa islet cells or LTM cells located in lamina II. These data reveal a differential distribution of 5HT axonal contacts on several types of neurons in laminae I and II, and support the view that at least a portion of the NRM modulation of dorsal horn neuronal responses to noxious stimulation is serotonergic and concentrated on the dendrites of nociceptive marginal and stalked cells.

230.8 A COMPARISON OF SUBSTANCE P AND SEROTONIN AXONAL CONTACTS ON IDENTIFIED NEURONS IN CAT SPINAL DORSAL HORN. M.J. Hoffert, V. Miletic, M.A. Ruda and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, Maryland 20205.

In previous studies we examined the distribution of serotonin axonal contacts on morphologically and physiologically characterized neurons in the superficial dorsal horn of the cat. The present study describes characteristics of substance P axonal contacts on similar neurons, and identifies properties that distinguish serotonin contacts from substance P contacts. The methods used have been described elsewhere (Miletic et al, Soc. Neurosci. Abst, this volume). Neurons intracellularly filled with HRP were immunocytochemically counter-stained either for substance P or for serotonin, and analogous cells were then compared. For direct comparison, some cells were divided into two portions and reacted separately for substance P and serotonin.

A variety of nociceptive dorsal horn neurons received substance P axonal contacts. On spiny marginal neurons, substance P axons preferentially contacted spine heads rather than dendritic shafts. On other spiny marginal neurons, serotonin axons preferentially contacted dendritic shafts. A lamina IV neuron received substance P contacts only on spine heads and small caliber dendritic side branches of a large dendrite in lamina I. Analogous portions of the cell, in adjacent sections stained for serotonin, received serotonin contacts primarily on the parent dendrites rather than on the small dendritic side branches. On relatively aspiny marginal neurons, substance P axonal contacts were present on dendritic shafts. These contacts were as numerous as those on the spines of spiny neurons. Most marginal neurons studied to date received about 5 substance P contacts per 100 µm of dendrite distributed evenly throughout their dendritic arbor. Some of these neurons also received a few somatic contacts. An occasional aspiny superficial neuron had numerous substance P contacts on its soma and proximal dendrites (about 50 contacts per 100 µm of dendrite). In contrast, the number of serotonin axonal contacts on marginal neurons was less variable with 10-15 contacts per 100 µm of dendrite.

Our findings indicate that morphologically different cell types in the superficial dorsal horn of the spinal cord receive both substance P and serotonin contacts. However, the distribution patterns of substance P and serotonin axonal contacts are different with respect to density and location. The more variable distribution of substance P is consistent with its multiple sources and presumed multiple functions.

230.9 TWO CLASSES OF NEURONS IN ANALGESIA-PRODUCING REGIONS OF THE ROSTRAL-VENTROMEDIAL MEDULLA. H. L. Fields, I. D. Hentall, G. Zorman*. Depts. of Neurology, Physiology and Neurosurgery, Univ. Calif., San Francisco, CA 94143.

Microstimulation of the nucleus raphe magnus (NRM) - nucleus reticularis paragigantocellularis (NRPG) region of the medulla inhibits the noxious heat-induced tail flick reflex (TFR) (Zorman et al, Brain Res. 219:137, 1981). Studies of neurons in this region have indicated that they may either be excited, inhibited or unaffected by noxious stimulation of their peripheral receptive fields. In order to clarify the relationship between a neuron's sensory response and its possible role in suppression of the TFR, we recorded cells in the medulla while eliciting the TFR and monitoring stimulus temperature.

Male Sprague-Dawley rats (300-350 gm) were anesthetized with 70 mg/kg pentobarbital i.p. Insulated stainless-steel microelectrodes (3-6 Megohms) were stereotaxically placed in the NRM/NRPG region. As the anesthesia level lightened, the TFR could be obtained at usual latency. The depth of the electrode was then adjusted so that suppression of the TFR was obtained at stimulation currents $\leq$20μA (400μsec, 50 Hz continuous trains). The depth was noted and the same electrode was used for single-unit extracellular recording of neurons from 1 mm above to 1 mm below the site. The receptive field of each cell was mapped. The relationship of its firing to tail temperature and to the occurrence of the TFR was examined by computer averaging of the spike activity (in a histogram) and stimulation temperature, with sweeps aligned by the onset of the tail flick. The threshold for TFR suppression was determined again at the end of each microelectrode penetration.

Two major classes of cell were found. The first type of cell which tended to be located more dorsally, in the region where thresholds for TFR suppression were higher, could be excited maximally by noxious stimuli over most of the body surface. Occasionally mechanical innocuous stimuli were also effective. This type of cell increased its discharge to noxious heating of the tail. The second cell type, more ventral in location, was often, but not always, inhibited by noxious stimuli (off-cells). In some off-cells, the reduction in discharge was apparently not a function of stimulation temperature but occurred at a fixed time prior to the TFR.

The observation that some "off-cells" are located at maximally effective sites for TFR suppression is consistent with the hypothesis that these cells must reduce their firing for the TFR to occur.

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230.10 BEHAVIORAL MODULATION OF TRIGEMINOTHALAMIC NEURONS IN THE MEDULLARY DORSAL HORN OF AWAKE MONKEYS. G.H. Duncan*, M.C. Bushnell, R. Dubner and L.F. He* (SPON: C. Bruce). Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, Maryland 20205.

In previous studies using awake monkeys we described medullary dorsal horn (trigeminal nucleus caudalis) neurons whose responses to thermal stimuli were modulated by behavioral variables. The present study investigated this behavioral modulation of thermal responsiveness in neurons that were shown to project from the medullary dorsal horn to the thalamus. Rhesus monkeys were trained to release a button when they detected either the onset of a noxious thermal stimulus (45^{o}-49^{o}C) or the termination of a warming stimulus (37^{o}-43^{o}C) presented via a thermode placed on the upper lip. The same monkeys were also trained in a visual task in which both light stimuli and thermal stimuli (37^{o}-49^{o}C) were presented. During that task, the monkeys were rewarded for button-release only when they detected the onset of a light and were never rewarded for detecting changes in the thermal stimulus. Single-unit activity was recorded in the medullary dorsal horn during the performance of the behavioral tasks. Projection neurons were identified by antidromic responses to stimulation of the ventral posterior medial thalamic nucleus. EMG activity was recorded from the obicularis oris muscle.

Thermal response thresholds of trigeminothalamic neurons ranged from 41^{o} to 45^{o}C, and stimulus-response functions were monotonic from threshold to 49^{o}C. Correlation of neuronal and behavioral data demonstrated that the discharge rate of thermosensitive projection neurons was modulated by behavioral factors. The neuronal response to behaviorally relevant thermal stimuli presented during performance of a task was greater than that produced by equivalent irrelevant thermal stimuli presented outside the task. Moreover, the neuronal responses to thermal stimuli presented within the thermal task were generally greater than the responses to equivalent thermal stimuli presented during the visual task. Comparison of neuronal and EMG activity confirmed that this task-specific enhancement of thermal responses was independent of mechanical influences.

The behavioral modulation of thermal responsiveness in sensory-discriminative projection neurons in the medullary dorsal horn suggests that ascending sensory information necessary for performance of a task is under the influence of descending controls related to the behavioral requirements of the task.

230.11 REDUCTION BY ELECTRICAL BRAINSTEM STIMULATION OF NON-NOXIOUS CUTANEOUS INPUTS TO SPINOCERVICAL AND SPINORETICULAR NEURONS. K.C. Kajander*, T.J. Ebner, and J.R. Bloedel. (SPON: W.W. Roberts). Depts. of Neurosurgery & Physiology, University of Minnesota, Minneapolis, 55455.

These experiments were designed to examine whether electrical stimulation of the periaqueductal gray (PAG) and the nucleus raphe magnus (NRM) modify the responses of ascending somatosensory systems to hair flick or other light cutaneous inputs. Although the PAG and NRM have been shown to modify the responses of dorsal horn neurons to peripheral noxious stimuli, it is generally assumed that these systems have little or no effect on non-noxious inputs. In adult cats anesthetized with alpha chloralose (60 mg/kg I.P.) a lumbar laminectomy was performed and a spinal cord oil bath was constructed. One bipolar stimulating electrode was placed above (medulla-C1 junction) and one below (C5-6) the spinocervical nucleus. Neurons recorded in the lumbosacral region of the spinal cord were identified as spinocervical cells if they were antidromically activated from only the lower cervical stimulus site and as spinoreticular units if they responded antidromically to both the upper and lower stimulus sites. A displacement controlled Ling vibrator was used to apply a cutaneous sinusoidal stimulus to the receptive field. Monopolar stimuli applied with metal microelectrodes to the PAG and NRM consisted of three to eight 500 Hz pulses with amplitudes between 75 and 400 uA. Post-stimulus time histograms time locked to the PAG or NRM stimulus but not to the peripheral input demonstrated a marked reduction of the impulse activity of both spinoreticular and spinocervical cells with stimulus intensities as low as 75 uA. The latency of this response ranged from 8 to 32 msec. The duration of reduced impulse activity evoked by either PAG or NRM stimulation ranged from 36-200 msec and was directly proportional to the intensity of the electrical stimulus. In a small percentage of spinoreticular cells the period of reduced impulse activity was preceded by an initial brief excitation lasting 12 to 36 msec. In a corollary set of experiments, the brainstem stimulus was timed to occur at a specific phase of the sinusoidal exteroceptive input. The cycle histograms revealed that the reduction in unit activity evoked from these brainstem sites is dependent upon the phase of the input at which the electrical brainstem stimulus occurs. Since these effects are phase dependent the action of these descending systems is not merely a simple reduction in the response amplitudes without regard to the temporal features of the input. More generally, these results show that both the PAG and NRM can modify the action of non-noxious inputs to two ascending somatosensory systems. Supported by NIH Grants #5R01-NS 13002 and #2R01-NS 09447-10.

230.12 BEHAVIORAL AND NEUROPHYSIOLOGICAL EVIDENCE FOR INVOLVEMENT OF NUCLEUS RAPHE MAGNUS IN ANALGESIA PRODUCED BY NEUROTENSIN. M.M. Behbehani and A. Pert. Dept. of Physiology, U. Cincinnati Coll. Med., Cincinnati, OH 45267 and Biological Psychiatry Branch NIMH, Bethesda, MD 20205.

Injection of neurotensin (NT) into the periaqueductal gray (PAG) of the rat produces analgesia. We have examined the mechanism of this analgesic effect of NT. In a series of behavioral experiments a cannula was implanted into the PAG of normals or animals that had received 10μg of 5-7 dihydroxytryptamine (DHT) or animals in which the nucleus raphe magnus (NRM) region had been lesioned by radio frequency. One week after surgery 1, 2 or 10 nmole of NT in 1μ lit of saline was injected in the PAG of normal animals. The lesioned animals received 10 nmole of NT. Animals were then tested in tail flick and hot plate tests. It was found that normal and those animals that had received 5-7 DHT in their NRM region showed significant analgesia after 10 nmole of NT. However, NT did not produce significant analgesia in animals with RF lesion. In physiological experiments 10 nmoles of NT in 1μ liter of saline were injected into the PAG and recordings were made from the NRM neurons. It was found that NT injected into the PAG caused a significant increase in the firing rate of the NRM neurons. The time to the onset of the response of NRM neurons was short and in most cases the firing rate increased with 10 seconds after NT injection into the PAG. The increase in firing rate remained elevated for as long as two minutes after injection of NT had stopped. Of the 20 cells studied in this part of the study, 14 cells were excited, 2 cells were inhibited and in 4 cells, NT injection into the PAG had no effect. In a second series of experiments, the effect of NT on PAG neurons was tested. Two barrel glass electrodes, one filled with Pontamine blue and one filled with 3 mM NT were used. A micropressure injection system was used to apply NT for 20 seconds. It was found that a large majority of the cells in the PAG responded to NT by excitation. Only a small percentage of cells in this area responded to NT by inhibition. The time to onset of response to NT was very short and in most cases baseline firing rate was changed within 8 seconds after NT application. The firing rate returned to baseline within 10 seconds after application of NT had stopped. It is concluded that the analgesic effect of NT when injected into the PAG is mediated by excitation of PAG cells, causing excitation of the NRM neurons which then cause analgesia by activation of the NRM-spinal inhibitory system. Since NT did not cause analgesia in animals that had received 5-7 DHT, it is concluded that serotinegic neurons of NRM region are not involved in this process.

231.1 IDENTIFICATION OF TUBEROINFUNDIBULAR LUTEINIZING HORMONE-RELEASING HORMONE NEURONS IN THE RAT THROUGH FLUORESCENCE DYE TRACING AND IMMUNOCYTOCHEMISTRY

M.O. Fraser*, M.J. Kelly and O.K. Ronnekleiv, Depts. of Physiology and Anatomy, Oregon Health Sciences University, Portland, Oregon 97201.

Previous electrophysiological and anatomical experiments have identified medial basal (MBH) hypothalamic neurons which project to the median eminence ("tuberoinfundibular" [TI] neurons). Recently, we have identified, through immunocytochemistry, luteinizing hormone-releasing hormone (LHRH) in the MBH of the rat, but we did not establish the exact projection of their axons (Kelly et al., Exp. Brain Res., in press). Presently, we have combined True Blue (TB) and Propidium Iodide (PI) injections into the median eminence (ME) with immunocytochemistry in order to identify the peptidergic distribution of TI neurons. The ME of nembutal-anesthetized female rats was exposed by the parapharyngeal approach. Microelectrodes (70 µm tip), which were filled with 5% PI or 2% TB, were visually placed in the ME. Single dye injections or contralateral injections of both dyes (4-6 µm, pressure-pulse injected) were made. The electrode was left in place with a slight back pressure for 1-2 hours before removing. After 3-6 hours survival under anesthesia, the rats were killed by decapitation and 400 µm thick sagittal slices were prepared and immersed in 4% paraformaldehyde in 0.1 M phosphate buffer for 75 min as previously described (Kelly and Ronnekleiv, Soc. Neurosci. Abstr. Vol. 7, p. 20, 1981). 16 µm sections were cut on a cryostat and the tissue processed for immunocytochemistry as previously described (Kelly and Ronnekleiv, op. cit.).We were able to inject dye into discrete areas in the ME with little or no diffusion to the contralateral side. The PI gave the greatest labeling of TI neurons, but the TB-labeled neurons survived the immunocytochemistry better. With injections of the dyes into the internal zone of the ME, dye-labeled LHRH neurons were observed in the arcuate and cell-poor zone. Injections into the rostral ME revealed dye-labeled LHRH neurons as far rostral as the medial preoptic area. Therefore, it is evident that both MBH and preoptic LHRH neurons project to the ME, but we do not know the functional differences between these two cell populations. Experiments are in progress to identify other TI neurons and to study the role of these LHRH TI neurons in the control of gonadotropin secretion. (Supported by NIH Grant NS 18989 and Oregon Medical Research Foundation).

231.2 TOPOGRAPHIC DISTRIBUTION AND EFFECTS OF KAINIC ACID LESIONS ON SOMATOSTATIN-LIKE IMMUNOREACTIVE MATERIAL IN THE RAT STRIATUM,

M.F. Beal, V.B. Domesick and J.B. Martin. Dept. of Anatomy and Neurology, Harvard Medical School, Boston, MA 02114.

The presence of somatostatin in the striatum is of particular interest since it may play an important role in degenerative diseases. Although previous studies have measured somatostatin-like immunoreactivity (SLI) levels in the striatum, we examined the possibility of a topographic distribution of SLI within sub-regions of the striatum. Somatostatin-like immunoreactivity (SLI) was detected by a specific radioimmunoassay in extracts from punches of rat striatum. Multiple regions were sampled from 1 mm thick sections of the striatum. The distribution of SLI was found to be topographically organized. Concentrations were highest in the nucleus accumbens and ventromedial striatum (4-5 pg/µg protein) and lowest in the dorsolateral striatum (1-2 pg/µg protein). Levels were significantly ($p<.05$) higher in the ventromedial striatum as compared to the dorsolateral striatum at each coronal level examined. Gel permeation chromatography of acetic acid extracts of whole rat striatum showed 5 distinct peaks of immunoreactive somatostatin. The two largest peaks coeluted with SS14 and SS28 and accounted for 72 and 12 percent of the total immunoreactivity respectively. To destroy striatal neurons, kainic acid was injected into the striatum in a dose of either 3.5 or 7.0 nmoles. Dopamine levels detected by HPLC were unaffected indicating that afferent terminals were preserved. Both dose levels resulted in an approximately 60% depletion of SLI on the ipsilateral side and a 25% reduction on the contralateral side. Histological inspection showed almost complete neuronal destruction. These findings suggest that about one half of the SLI in the striatum can be localized to intrinsic neurons. The remainder of the SLI may be contained in afferent fibers since the topographic distribution of somatostatin corresponds to that of several inputs from the limbic system.

231.3 PRENATAL ONTOGENY OF SOMATOSTATIN (SOM)* IMMUNOREACTIVE ELEMENTS IN THE RAT SPINAL CORD.

R.H. Ho. Dept. of Anat., Coll. of Med., Ohio State Univ., Columbus, OH 43210.

Prenatal rats of gestational ages E12-22 were immersion fixed with Zamboni's solution and transverse cryostat sections of their spinal cords processed by Sternberger's PAP technique. SOM was first detected in the spinal cord at E13 when it was observed within presumptive perikarya of the basal plate. In addition, at E13 and 14, SOM was demonstrable in neurons of dorsal root ganglia whose SOM immunoreactive central processes approximated the dorsal lateral surface of the spinal cords. At E14, SOM fibers were first detectable in the ventral funiculi. At E15, SOM elements resembling cell bodies first appeared in the superficial laminae of the dorsal horn while SOM fibers were present in the lateral funiculus. By E16, the gray matter contained widely dispersed SOM perikarya of various shapes and sizes, but few SOM varicosities were present. At E18 SOM fibers were present in the dorsal funiculus. By E20, a moderate density of SOM varicosities became obvious in the superficial laminae of the dorsal horn. SOM was widespread in the E22 spinal cord being most numerous within the superficial laminae of the dorsal horn. The outer portion of this region contained SOM varicosities whereas the inner portion also contained small perikarya. SOM perikarya of various shapes and sizes were widely dispersed throughout the gray matter ventral to the superficial laminae, with a slightly higher density found in the region ventrolateral to the central canal. A moderate density of SOM fibers was present in the lateral funiculus, whereas only a small number were located in the dorsal and ventral funiculi. The function of the SOM immunoreactive cell bodies in the spinal cord and dorsal root ganglia during development remains to be determined. However, their presence early in development suggest that they may play a role in the ontogeny of other spinal systems. (Supported by NIH NS-10165.)

*A substance's immunoreactivity is referred to by its name.

231.4 MAPPING OF IMMUNOCYTOCHEMICALLY IDENTIFIED SOMATOSTATIN-CONTAINING NEURONS IN THE PIGEON BRAIN.

M.L. Berk, R.H. Ho and J.A. Finkelstein. Program in Human Anatomy, Northeastern Ohio Univs. College of Medicine, Rootstown, OH 44272, and Department of Anatomy, Ohio State University, Columbus, OH 43210.

The distribution of somatostatin (SOM)*-containing neuronal perikarya in colchicine treated brains of the pigeon (Columba livia) was investigated. Frozen sections processed by the PAP technique of Sternberger contain SOM perikarya from caudal medullary levels to rostral telencephalic levels. In the medulla, immunoreactive SOM perikarya are observed in the following regions: 1. numerous cells in all subdivisions of the inferior olivary nucleus; 2. nucleus subtrigeminalis; 3. nucleus solitarius; 4. nucleus cuneatus externus; and 5. ventrolateral reticular formation. Immunoreactive perikarya are also found in the ventrolateral reticular formation of the pons. At midbrain levels, numerous, large, multipolar SOM cells occur in the central gray and nucleus intercollicularis. Fewer SOM cells are observed ventral to the midbrain central gray in the reticular formation. The interpeduncular nucleus contains small, immunoreactive cells. Small cells containing SOM are also present in a region dorsal and ventral to non-immunoreactive fibers entering the tectal commissure of the midbrain. At the hypothalamo-midbrain junction, large, darkly stained neurons are located in the stratum cellulare internum between the cerebral aqueduct and the infundibular recess of the third ventricle. A few immunostained cells occur in the lateral hypothalamus and lie adjacent to SOM fibers which course into the external layer of the median eminence. Prominent, multipolar SOM cells are seen in the ventral part of the posteromedial hypothalamic nucleus, while a few small SOM cells are observed in and lateral to the tuberal nucleus. At anterior hypothalamic levels, dense, bipolar, periventricular SOM cells have their processes oriented perpendicular to the third ventricle. In the telencephalon, the hippocampal area contains many, darkly immunostained, multipolar cells. Small, weakly stained cells are observed in the medial septal nucleus while a few moderately stained cells occur lateral to the lateral ventricle in various telencephalic regions. Immunoreactive neurons are observed in many telencephalic areas, particularly at the dorsal, ventral, and lateral surfaces of the brain. The location of SOM neurons in the pigeon will be compared to the rat.

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*A substance's immunoreactivity is referred by its name.

231.5 EFFECTS OF LESIONS OF THE SUPRACHIASMATIC NUCLEUS ON VASOPRESSIN IMMUNOREACTIVITY IN THE RAT FOREBRAIN. S.J. Tallaksen*, S.A. Joseph and K.M. Knigge. Neuroendocrine Unit, University of Rochester, Rochester, NY 14642.

Projections of parvocellular vasopressinergic (AVP) neurons were examined immunocytochemically in the rat following unilateral lesion of the suprachimastic nucleus (SCN). Male rats (160-180 gm) were placed in a stereotaxic apparatus and a stainless steel electrode lowered to the level of the SCN, using an angular approach from the contralateral side. A discrete lesion of the SCN was obtained by applying an anodal direct current of 0.3-0.4 mA for 15-20 seconds. Sham-operated animals underwent identical surgical procedures, but no current was passed. Following a survival period of 7 days animals were perfused through the ascending aorta with Bouin's fixative. Brains were removed and 50 µm coronal sections cut on a vibratome. Sections were incubated in Ferring anti-AVP at a dilution of 1:8000 and processed for immunocytochemistry according to the unlabeled antibody enzyme method. The distribution of AVP neurons and fibers in control animals has been extensively described. Unilateral destruction of 80-100% of the SCN resulted in a marked ipsilateral reduction or disappearance of fine-caliber immunopositive fibers in most regions of the forebrain. Diminished fiber distribution was observed throughout the periventricular zone of the preoptic region, hypothalamus and thalamus. Fibers coursing ventrolaterally over the optic chiasm toward the supraoptic nucleus were absent. In addition, there was a reduction of AVP-immunoreactivity within the medial preoptic and parvocellular paraventricular nuclei. These results indicate that the efferent outflow of AVP-immunoreactive cells in the SCN to forebrain structures is predominantly ipsilateral.
(Supported by USPHS grant 5T32 GM07136-07 and NINCDS Program Project NS15345.)

231.6 TRANSMITTER-SPECIFIC PROJECTIONS OF THE RAPHE-SPINAL SYSTEM. R.M. Bowker*, M.C. Sullivan*, J.F. Wilber* and J.D. Coulter. Marine Biomedical Institute, University of Texas Medical Branch, 200 University Boulevard, Galveston, Texas 77550 and Department of Internal Medicine, Louisiana State University Medical Center, New Orleans, Louisiana 70112.

Immunocytochemical studies have demonstrated a rich innervation of the spinal cord by fiber terminals containing serotonin(5HT) and various of the neuropeptides, including substance P(SUB P), thyrotropin-releasing hormone(TRH) and leucine and methionine enkephalin(ENK). These terminals originate, wholly or in part, from neurons of the brainstem raphe nuclei. In the present study, descending neurons containing serotonin and various of the neuropeptides that project to the spinal dorsal and ventral horn were identified using a combination of retrograde axonal tracing and immunocytochemical methods along with funicular lesions of the spinal cord. Anesthetized rats received injections in the spinal cord with horseradish peroxidase(HRP) or wheat germ agglutinin conjugated to HRP(WGA-HRP) to localize the descending 5HT and peptidergic projections. Tissue sections were then processed for HRP histochemistry and routine immunocytochemistry (Brain Res. 211: 412-417). In the medulla, the origins of spinally projecting immunoreactive neurons stained for 5HT, SUB P, TRH and ENK were localized in the nucleus raphe obscurus, raphe pallidus, raphe magnus, and adjacent parts of the reticular formation. Following various spinal cord funicular lesions neurons of the caudal raphe cell groups were found to have descending projections in the ventral half of the spinal cord, while neurons of the anterior raphe nuclei traverse the dorsolateral funiculus. Microinjections of HRP, or WGA-HRP, into various regions of the spinal gray matter were also used to identify the locations of those serotonergic and peptidergic raphe neurons projecting to the spinal dorsal and ventral horn. Analyses of serotonergic and peptidergic immunoreactive terminal staining in the ventral horn and intermediate grey regions of the spinal gray matter following funicular lesions were also performed. Together the results indicate that the descending serotonergic and the different descending peptidergic pathways provide innervation of both the dorsal horn and the ventral horn of the spinal cord. Supported by NS12481.

231.7 DISTRIBUTION OF SUBSTANCE P NEURONS IN THE HYPOTHALAMUS OF THE RHESUS MONKEY AS DETERMINED BY IMMUNOCYTOCHEMISTRY. O.K. Ronnekleiv and M.J. Kelly., Departments of Anatomy and Physiology, Oregon Regional Primate Center, Oregon Health Sciences University, Portland, Oregon 97201.

Substance P (SP) has been found to stimulate prolactin secretion in different mammals including the Rhesus monkey. The stimulatory effect has been suggested to occur at hypothalamic level (Rivier et al., Endocrinology 100-751, 1977) and/or at pituitary level (Vijayan and McCann, Endocrinology 105:64, 1979). In the monkey, a very dense plexus of fluorescent SP fibers has been demonstrated in the external layer of the median eminence. The origin of these fibers, however, is uncertain. In order to further understand the action of SP on prolactin secretion, we decided to delineate the location of the SP positive perikarya in the monkey hypothalamus. 3 adult female and 3 adult male Rhesus monkeys were perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). The hypothalamus was dissected and kept overnight in phosphate buffer containing 10% sucrose. Sagittal and coronal cryostat sections were cut, mounted on gelatinized slides and kept frozen until processed by the PAP method of Sternberger for light microscopy as described previously (Kelly et al., Exp. Brain Res. in press). The SP antiserum (courtesy of Dr. Eskay), used at a 1:1000 dilution, stained neurons in the female and male monkey hypothalamus. The perikarya were round to fusiform with one to two processes extending from the cell soma. The majority of the cells were located in the arcuate-median eminence (A/M) region. Very few cells were seen in the rostral while the density of neurons increased towards the middle and caudal region of the A/M. Single SP-positive fibers could be followed dorsally along the ventricle; however, the majority of SP positive fibers coursed medially and ventrally into the infundibular stalk where a dense plexus was found in the intermediate lobe. SP-positive fibers were also found to extend into the neural lobe of the infundibular stalk. These data demonstrate that SP-positive perikarya are located in the mediobasal hypothalamus of the monkey and indicate that these neurons project into the infundibular stalk. (Supported by NIH Grants NS 18848 and NS 18989).

231.8 SUBSTANCE-P IMMUNOREACTIVITY IN LARGE DENSE GRANULES IN LAMINA II OF THE CAT DORSAL HORN. H. David Coulter. Department of Anatomy, University of Minnesota, Minneapolis, Minnesota 55455.

Fresh tissue from the dorsal horn of the cat spinal cord was frozen at liquid helium temperature, dried in a vacuum, fixed with OsO_4 vapor, and infiltrated with epoxy resin. Blocks were serially sectioned to obtain repeating sequences of paired 0.25 µm sections for light microscopy and paired 0.10 µm sections for electron microscopy, one section in each pair for the experimental stain and the other for an absorption control. The 0.25 µm sections were stained with 1:100 dilutions of an antibody to substance P (S-P) (Raymond Ho) followed by FITC protein "A" for fluorescence microscopy. Absorption controls were incubated with 10 µg S-P/ml. Microscope images were taken from a TV monitor connected to an image intensified TV camera. Fluorescence images using reflected light were compared to phase and Nomarski contrast images to determine the morphological correlates to the positive staining. Small regions of tissue positive for S-P, varying in diameter from 0.3-1.5 µm, were found primarily in lamina II of the dorsal horn. Nuclei, areas of cytoplasm around nuclei, and capillaries were all negative. Absorption controls were negative.

For ultrastructural comparisons, alternate pairs of 0.10 µm sections were treated with 1:10,000 dilutions of the S-P antibody followed by the PAP method for electron microscopy. Absorption controls were carried out with as little as 100 pg S-P/ml. Most large dense granules in lamina II were positive. Nuclei, nucleoli, plasma membranes, mitochondria, myelin, background cytoplasm, small clear vesicles, and synaptic densities were negative. The absorption controls were similar to the experimentals, except that all large granules were negative. Comparing the electron micrographs with the light microscope images revealed that the positive regions of fluorescence corresponded to axon terminals in the neuropil of lamina II. These results indicate that substance-P immunoreactivity in the dorsal horn of the spinal cord is probably limited to the confines of large vesicles.

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231.9 LOCALIZATION OF CHOLECYSTOKININ (CCK) IN NUCLEUS TRACTUS SOLITARIUS (NTS) OF THE RAT AND ITS POSSIBLE VAGAL ORIGIN. J.Z. Kiss*, T.H. Williams, M.C. Beinfeld*, and M. Palkovits*, Department of Anatomy, University of Iowa, Iowa City, IA 52242; and Neuroendocrine Unit, Lab. Clin. Sci. NIH, Bethesda, MD 20205.

Gastrointestinal tissue and the central nervous system both contain a CCK-like substance. In the brain this octapeptide has been identified in neurons, and ultrastructural recognition of nerve terminals showing CCK-like immunoreactivity associated with synaptic contact sites would provide evidence to support a neurotransmitter function. Since the nucleus of the tractus solitarius is a site of termination of afferent fibers of cranial nerves V, VII, IX, and X, and because this nucleus contains high concentrations of CCK, we decided to address the ultrastructural immunolocalization and the possible source of the CCK-like immunoreactivity in the NTS.

In control animals, radioimmunoassay confirmed that the peptide is present in the NTS in a relatively high concentration. Using light and electron microscopic immunocytochemistry, CCK-like immunopositive nerve fibers and terminals were demonstrated in the NTS. Synapses were observed between immunoreactive boutons containing labeled round dense cored vesicles and unlabeled dendrites. Most of the synapses observed were symmetric (or Gray type II). No CCK-positive cell bodies, and very few lightly stained CCK-immunoreactive dendrites were observed in the nucleus.

The origin of the CCK containing terminals was determined by 1) transecting the major neuronal afferents to the NTS and then measuring the CCK content of the nucleus; 2) injecting colchicine to enhance cell-body visualization. The following results indicate that CCK in the NTS is of extrinsic, most probably vagal, origin: a) transection of the major neuronal afferents (via the solitary tract and/or some lateral pathway) resulted in a complete loss of detectible CCK in the NTS, as evaluated by radioimmunoassay; b) axon fragments of the intracranial vagus destined for the NTS were immunostained for CCK; c) CCK immunostaining is mainly localized in nerve terminals in the NTS. Immunostained dendrites or cells (after colchicine treatment) occured only in very few numbers.

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231.10 LOCALIZATION OF FMRF-NH_2-LIKE IMMUNOREACTIVITY IN AUTONOMIC REGIONS OF THE BRAINSTEM AND SPINAL CORD OF THE RAT C.A. Sasek*, R. Elde and V.S. Seybold (Spon: M. Wessendorf). Department of Anatomy, Univ. of Minnesota Medical School, Minneapolis, MN 55455

Antisera raised against the molluscan cardioexcitatory peptide (FMRF-NH_2) have recently been demonstrated to recognize a peptide within the central nervous system. The amino acid sequence of the peptide localized by these antisera in mammals is not known, but it is presumed to be larger than the molluscan tetrapeptide. The present study extends earlier findings in which FMRF-NH_2-like immunoreactivity was observed in widespread areas of brain and spinal cord.

In order to morphologically characterize neuronal systems containing the FMRF-NH_2-like peptide, antisera against FMRF-NH_2 were generated in rabbits. Ten µm frozen sections of brain and spinal cord taken from normal or colchicine treated rats perfused with phosphate buffered 4% paraformaldehyde (pH 6.5) followed by borate buffered 4% paraformaldehyde (pH 11) were processed for immunoflourescence. The tissue was incubated with FMRF-NH_2 antisera (1/400) or FMRF-NH_2 antisera (1/400) preabsorbed with FMRF-NH_2 (10µg/ml).

In the brainstem FMRF-NH_2-like immunoreactivity was observed in an aggregration of fibers in the lateral parabrachial nucleus. Cell bodies were seen in the commissural and medial subdivisions of the nucleus of the solitary tract. At all levels of the spinal cord fibers were concentrated in laminae I and II, and scattered fibers were present through deeper regions of the dorsal horn. At the lumbar level a high density of fibers was also present around the central canal. At sacral levels fibers were concentrated in the medial aspect of the dorsal horn, n. intercalatus disseminata and n. intermediolateralis sacralis pars principalis and pars ala ventrallis. In addition, scattered fibers were seen in the ventral horn and bundles of fibers could be followed from the ventral gray matter to ventral white matter. In addition to immunoreactive fibers a large number of cell bodies were observed in n. intercalatus disseminata. Adjacent sections incubated with an absorption control showed no staining.

These results are consistent with the hypothesis that an FMRF-NH_2-like peptide is localized within certain subpopulations of neurons in mammals. The immunolocalization of this peptide in autonomic structures of the brainstem and spinal cord provides another neurochemical marker that can be used to investigate neuronal circuits underlying central autonomic regulation.

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231.11 LOCALIZATION OF NEURONS CONTAINING DELTA-SLEEP-INDUCING PEPTIDE IN THE RAT BRAIN. S.C. Feldman and A.J. Kastin*. Dept. of Anatomy, NJ Medical School, Newark, N.J. 07103 and V.A. Medical Center and Tulane University Medical School, New Orleans, LA 70146.

Delta-sleep-inducing peptide (DSIP), the nonapeptide found to be related to the occurrence of sleep, has been implicated in some non-sleep processes including thermoregulation. DSIP-like immunoreactivity has been demonstrated by RIA in several areas of the rat brain (Brain Res. Bull., 3:691, 1978) but the distribution of DSIP-containing neurons remains unknown. We now report the occurrence of DSIP-like material in neurons from brains of colchicine-treated and normal rats. Brains from all animals were fixed in Bouin's solution, sectioned and DSIP localized in neuronal elements by the PAP technique of Sternberger; the antibody to DSIP was used previously in the RIA study. All immunoprecipitate in tissue sections was specific for DSIP since preadsorption of the antibody with DSIP eliminated staining in neuronal elements. By immunocytochemistry, DSIP neurons were found to have a widespread distribution in the CNS. There was no apparent difference in the number or location of DSIP-containing neurons after colchicine treatment. The majority of DSIP perikarya were localized in the brainstem: reticular formation, raphe nuclei, N. tractus solitarius, N. medulla oblongata, superior trigeminal N., N. tractus spinalis oralis and N. centralis caudalis oralis. DSIP-containing perikarya were also found in the superior and inferior colliculi, N. medial lemniscus, superior olivary N., N. mesencephalic tract of V, cortical and medial amygdaloid nuclei, caudate/putamen, diagonal band of Broca, hippocampus and cerebral cortex. In the hypothalamus, DSIP was present mainly in the lateral hypothalamic area; fewer neurons were found in dorsal and ventral-medial nuclei, arcuate N., and preoptic area. DSIP was generally absent from fiber tracts including the median eminence. In thick (75um) sections from colchicine-treated animals the peptide could be demonstrated in proximal processes as well as in neuronal perikarya. In general, immunoreactive DSIP-like material was found by RIA in each area in which DSIP was localized by immunocytochemistry. The presence of DSIP neurons throughout the brainstem is consistent with a role for the peptide in sleep. Neurons containing DSIP-like immunoreactivity is present in areas known to contain other peptides and putative transmitters such as the brainstem, cortex, hippocampus and hypothalamus suggesting that DSIP could play a role in many functions by modulating the release of other transmitters. Supported in part by a grant from the Foundation of UMDNJ to S.C.F. and the Veterans Administration to A.J.K.

231.12 α-MSH IS A POSSIBLE NEUROTRANSMITTER IN THE RAT VAS DEFERENS. T. Smock*, H. L. Fields, L. Jan and Y.-N. Jan. (SPON: I. Hentall). Depts. of Physiology and Neurology and Neuroscience Program, University of California, San Francisco, San Francisco, CA 94143.

The pro-opiomelanocortin products ACTH, α-MSH and β-endorphin (β-E) appear to inhibit autonomic neuromuscular transmission in the rat vas deferens (RVD). Although ACTH and α-MSH (ID50=9 nM) are more powerful than β-E (ID50=90 nM) by an order of magnitude they do not appear to exert their effect by means of an opiate receptor.

Since the liminal dose for the neuropeptides in this system exceeds the concentration found in plasma, we surmised that the autonomic fibres themselves contained the peptides, possibly together with conventional transmitters. As an initial test of this hypothesis RVDs were fixed in 4% formalin, washed overnight in 5% sucrose, sectioned on a freezing microtome at 10 µm and processed for immunocytochemistry.

The primary serum (Immuno Nuclear) was directed against α-MSH and had little cross-reactivity with CLIP (ACTH 18-39). At 1:200 serum preabsorbed with synthetic α-MSH (Peninsula Labs, 100 µg/ml) showed no reactivity with material in the RVD, but unabsorbed sera reacted heavily as indicated by the bright fluorescence of the secondary rhodamine-labelled goat-anti-rabbit serum (1:50) that was bound to the tissue.

Immunoreactivity was apparent in a large number of the post-ganglionic fibres investing the smooth muscle layers. This suggests that the antigen co-exists with either acetylcholine or norepinephrine since pharmacological manipulations demonstrated that both of these systems innervate the duct.

β-E and β-LPH have been shown to exist in rat testes and seminal vesicle, but none appeared in the RVD (Sharp et al, BBRC 95:618, 1980). This may be due to the small tissue volume used in the RVD extracts or to differential processing of pro-opiomelanocortin as seen in the pituitary and brain.

Since the RVD is a simple preparation that may employ the peptides as transmitters it seems that it could be an excellent system for the study of their functional interaction.

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232.1 PARALLEL PROCESSING OF BINOCULAR DISPARITY IN THE CAT'S GENICULO-CORTICAL PATHWAY, John D. Pettigrew and Bogdan Dreher*, National Vision Research Institute, 386 Cardigan Street, Carlton, Victoria 3053 and Department of Anatomy, University of Sydney, New South Wales 2006, Australia.

Three parallel streams of information-processing have been traced in the cat from X-, Y- and W-type retinal ganglion cells to Areas 17(X, Y & W), 18(Y) and 19(W). In the present investigation we have examined the role played by these three areas in the processing of binocular retinal disparity. We were stimulated by the hypothesis of Levick, that Y-cells might be specifically concerned with the processing of more convergent disparities than those processed by X-cells. Similarly, recent evidence on the nasotemporal decussation patterns of the different ganglion cell classes suggests that the epsilon subclass of W-cells might be concerned with the analysis of divergent disparities in comparison with X-cells. (Leventhal, pers.comm.).

We measured binocular receptive field disparities in paralyzed, anaesthetized adult cats. The tapetal reflection technique was used to monitor residual eye movements and to provide a map, for each eye, of the retinal blood vessels which could later be compared with retinal whole mounts stained to reveal the area centralis. In this way, receptive field disparities from different cortical regions could be compared, not only with each other, but with reference to the visual axis defined by the area centralis of each eye.

Cells of Area 19 had receptive field disparities which were significantly more divergent than those of cells in Areas 17 and 18. Referred to the area centralis the mean receptive field disparity in Area 19 was $-0.5^{o} \pm 0.8^{o}$ S.D. Mean receptive field disparity in Area 18 was convergent ($+3.5^{o} \pm 1.2^{o}$ S.D., or $+4.6^{o} \pm 2.0^{o}$ S.D. if data in the "transcallosal" region at the 17/18 border are included). In Area 17 mean receptive field disparity was also convergent with respect to the visual axis ($+2.0^{o} \pm 0.5^{o}$ S.D.). At the level of the lateral geniculate nucleus there was a significant difference between disparities measured at the A-A1 border ($+2.1^{o} \pm 0.3^{o}$ S.D.) and at the C1-C2 border ($-0.2^{o} \pm 0.2^{o}$ S.D.). This difference is consistent with the idea that there is a subclass of W-cells projecting to Area 19 via the C-laminae which codes more divegent disparities than the X-cell pathway through the A-laminae to Area 17.

Our results support a tripartite arrangement of disparity-processing within the geniculo-cortical pathway, where convergent and divergent disparities are handled by the Y(alpha) component of Area 18 and the epsilon component of Area 19 respectively, while the fixation plane is processed by the X-(beta) component of Area 17.

232.2 RECEPTIVE FIELD ORGANIZATION OF AREA 18 NEURONS, C.E. Sawyer* and L.A. Palmer. Dept. of Anatomy, School of Medicine, Univ. of Pennsylvania., Phila., Pa. 19104

We have examined the detailed receptive field (RF) organization of over 130 neurons in area 18 of the cat. Cells' responses to static stimuli were examined using the response plane technique of Stevens and Gerstein[1]. We anticipated that RFs in area 18 would differ significantly from those in area 17 since area 18 receives direct Y input from the LGN, but unlike area 17, no direct X input. Since simple cells in area 17 are known to exist in X-like and Y-like forms (Mullikin et al., Soc. Neurosci. Abst., Vol. 7 p.356, 1981), we anticipated that simple cells in area 18 would all be Y-like.

Our expectations were largely confirmed. The RF organization of simple cells in area 18 closely resembled that of Y-like simple cells in area 17. Individual excitatory regions within the RF, however, were about 3 times as large as those in 17. This class accounted for less than 25% of the cells sampled.

Based on their spatio-temporal organization, the majority of area 18 RFs defied classification as simple or complex as those terms are used in area 17. While RF classification in area 17 was based primarily on the spatial distribution of excitation and inhibition, in area 18 it was the temporal RF organization which was most striking.

Two broad classes of RFs were seen in addition to the Y-like simple cells. One class was defined by extremely transient (on the order of 10 msec.) responses to light on and/or off. Frequently these cells had repetitive transient peaks in their response. These repetitive peaks had a consistent temporal separation within circumscribed regions of RFs. The temporal separation of repetitive peaks varied from cell to cell with a range of 20 to 70 msec.

The second general class of RFs was defined by a mixture of sustained and transient components. The spatio-temporal organization of these cells was very complicated. Frequently the sustained on and off regions were spatially non-overlapping while the transient components extended across the entire RF. The response latency of the transient components often varied by up to 50 msec. in different regions of a single RF.

These data suggest that the exquisite spatial organization seen in area 17 RFs may be paralleled by the equally detailed temporal organization of area 18 RFs.

[1] Stevens, J.K. & G.L. Gerstein, J. Neurophysiol. 39:213-238 (1976)

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232.3 AN ADDITIONAL RETINOTOPICALLY ORGANIZED VISUAL AREA (PS) WITHIN THE CAT'S POSTERIOR SUPRASYLVIAN SULCUS AND GYRUS. B. V. Updyke Dept. of Anatomy, LSU Medical Center, New Orleans, LA 70112.

In the course of studies of the visually responsive cortex of the cat's posterior suprasylvian sulcus and gyrus, another partial representation of the visual hemifield was encountered adjoining visual areas DLS, VLS, 21b and 20b as described by Palmer et al. (J. Comp. Neurol., 177(1978):237-256) and Tusa and Palmer (J. Comp. Neurol., 193(1980):147-164). Since this new area conforms to the region denoted PS by Heath and Jones (Ergeb. Anat. Entwickl.-Gesch., 45(1971):1-64), their nomenclature is retained here.

In preparations anesthetized with chlorolose, this inferior region of the posterior suprasylvian sulcus and gyrus is consistently visually responsive, and a partial representation of the lower quadrant of the contralateral hemifield can be identified. Receptive field centers range in elevation between $+5^{o}$ and -30^{o}, and in azimuth between 0^{o} and 25^{o}; both elevation and azimuth are systematically represented. The representation of 0-elevation adjoins DLS, VLS and 21b, and negative elevations are represented more inferiorly. Representation of 0-azimuth is located anteriorly, with increasing azimuth represented posteriorly.

In order to establish that PS is a separate visual area and not simply a previously overlooked portion of an adjacent area, it was necessary to examine its anatomical connections. In these experiments, PS was first identified by electrophysiological mapping, and then injected with HRP or ^{3}H-proline. Following HRP injections into PS, significant numbers of labeled neurons are found in lateral posterior zones LP1 and LPi, and in the rostral part of area 19 and the adjacent region of area 7. Injections of ^{3}H-proline into PS result in terminal labeling within zone LPi, and within an extensive array of cortical visual areas. Included among these cortico-cortical projections is a distinctive projection to areas 7, 19, PMLS, PLLS, AMLS, ALLS, and SVA which occurs as continuous strata of terminal labeling within layers I and VI. This unusual pattern of cortico-cortical projections clearly distinguishes PS from adjacent visual areas, and further suggests that PS plays a unique role in modulating the activity of a diverse collection of additional visual areas.

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232.4 THALAMOCORTICAL RELATIONS OF A VISUAL AREA IN THE LATERAL BANK OF THE LATERAL SULCUS IN THE CAT. R. R. Marcotte and B. V. Updyke. Dept. of Anatomy, LSU Medical Center, New Orleans, LA 70112

The observation that area 7 of the middle suprasylvian gyrus interconnects with the rostral region of the lateral posterior (LP) complex in an unusual manner (Berson and Graybiel, Brain Res. 147:139, 1978) prompted us to examine the visual responsiveness and potential retinotopic organization and to reexamine the connections of this area. A limited portion of area 7 within the lateral bank of the lateral sulcus was mapped in cats anesthetized with chlorolose. Within this area, a partial representation of the lower quadrant hemifield was identified. Penetrations through the lateral wall showed sequences in which large receptive fields were encountered initially followed by progressively smaller fields, clustered around the center of gaze, as the electrode approached the fundus. In general, the internal organization of this area resembled that of area 19 along the medial wall of the sulcus.

Following the completion of mapping, HRP was injected within the mapped area. Labeled cells were found to be clustered dorsally in two short bands within the rostral pole of the LP complex. Cells in the lateral band were confined to the rostral portion of the pulvinar zone. Labeled cells in the medial band were located in an area which Updyke (J. Comp. Neurol., 173:81, 1977) has called the lateral LP zone (LP1). Berson and Graybiel, alternatively have suggested that this area constitutes a separate nucleus apart from the LP complex which they called the caudal division of the lateral intermediate nucleus (LIc).

It is interesting to note that the two columns of labeled cells in the present study align and partially overlap with more extensive columns of labeled cells within the pulvinar and LP1 zones resulting from injections of HRP into comparable representations of the lower field quadrant in area AMLS (Marcotte and Updyke, Anat. Rec., 199:160, 1981). In this previous study, columns of labeled cells were found to extend through both the caudal and rostrodorsal regions of the LP1 zone. Thus this rostrodorsal region which Berson and Graybiel have designated as LIc shares both common lines of isorepresentation and common connectional patterns with the remainder of zone LP1. These results indicate that despite differences in connectional patterns between the rostrodorsal and caudal regions of the LP1 zone, the organization of the rostrodorsal region is consistent with a continuous system of isorepresentation which extends throughout the entire LP1 zone.

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232.5 LIGHT AND EM ANALYSIS OF CYTOCHROME OXIDASE-RICH ZONES IN THE PRESTRIATE CORTEX OF SQUIRREL MONKEYS. M. Wong-Riley and E. Carroll*. Dept. of Anat., Med. Coll. of Wis., Milw., WI 53226

Distinct patterns of cytochrome oxidase (C.O.) activity have been found in the striate cortex of primates (Horton & Hubel, '80; Humphrey & Hendrickson, '80; and our own observations). We sought to determine whether the prestriate cortex exhibited unique patterns of activity which may provide insight into the functioning of the area. At the 17/18 border of squirrel monkeys, the intense C.O. staining of laminae IVA and IVC of the striate cortex abruptly ended and a new pattern of activity continued for 4-6mm along the dorsolateral visual cortex. Within this prestriate area, periodic puffs of high C.O. activity appeared in laminae 3 and 2, with the highest activity in lower 3(3B) extending slightly into upper 4. There was a faint hint of a columnar pattern in that lam 4 and especially 5 below the puffs were slightly more reactive than adjacent areas. At times, the staining in 5 could be dissociated into a thin band in upper 5(5A) and another one between 5 and 6. Lam 1 was also quite reactive. In horizontal sections, the reactive puffs measured approximately 640μm in diameter, with a center-to-center spacing of about 1100μm. The interpuff non-reactive zones had a diameter of about 300μm, with a center-to-center spacing of 1100μm. Both reactive and non-reactive stellate and pyramidal cells were found between lam 2 and 6, but the frequency of reactive neurons were slightly higher within the puffs. At the EM level, the intensely reactive cells tended to be medium sized neurons with severely indented nuclei and darker cytoplasm filled with reactive mitochondria. Reactive pyramidal neurons likewise had a large number of mitochondria. The majority of small stellates with scanty cytoplasm and few mitochondria were non-reactive. They received none or 1 to 2 symmetrical synapses on their cell bodies in a single plane of section. Thus far, asymmetrical axo-somatic synapses were found only on reactive neurons. Within the puffs, dendritic mitochondria comprised up to 50% of the total mitochondrial population, and the majority of them were moderate to highly reactive. Between 1/3 to 1/2 of the mitochondria in axonal profiles were reactive, the frequency being slightly higher in profiles with flattened vesicles than those with round ones. Myelinated axons could be reactive or non-reactive, whereas glial cells were mainly non-reactive.

Our results indicate that organized zones of high metabolic activity occur in the prestriate cortex, as in the striate cortex. Both regions show puffs of C.O. reactive zones in the supragranulayers. The location and distribution of reactive puffs in the prestriate cortex correspond closely to those of pulvinar-prestriate terminations reported by Curcio and Harting ('78) in the squirrel monkeys. (Supported by NIH grant NS18121).

232.6 PROCESSING OF COLOR, FORM AND DISPARITY IN VISUAL AREAS V2 AND VP OF VENTRAL EXTRASTRIATE CORTEX IN THE MACAQUE. A.Burkhalter* and D.C.Van Essen. Biology Division, Caltech, Pasadena, CA 91125.

The ventral posterior area (VP) in the macaque lies in extrastriate cortex anterior to the ventral half of V2, and it contains a representation of only the upper part of the visual field. It differs from V3, which borders the dorsal half of V2, in its absence of direct input from V1, its pattern of callosal inputs, and its myeloarchitecture (Newsome et al., Soc.Neurosci.Abstr.6: 580, 1980). To learn more about functional specialization in ventral occipital cortex, we have recorded from single cells in VP and ventral V2 and have compared responses to stimulus orientation, direction, binocular disparity and color.

Quantitative analyses were made on 101 neurons, 57 assigned to V2 and 44 to VP, based on the topographic organization of receptive fields. The most striking result was the finding of a high incidence of color-sensitive neurons in V2 and VP. In both regions about half of the cells studied were sensitive to changes in the wavelength of equal luminosity stimuli. Many of these responded only to a narrow portion of the visible spectrum and far less if at all to white light of equal luminosity. Responses were classified as color-biased (V2:6/37, VP:9/41), color-biased with opponency (V2:11/37, VP:9/41), color-opponent (V2:1/37, VP:3/41) and non-color-coded (V2:18/37, VP:16/41). A few cells (V2:1/37, VP:4/41) preferred black stimuli and were categorized separately.

In both regions there were many cells which were selective for stimulus parameters other than color. A similar percentage were orientation selective in V2 (20/26) and in VP (22/33). The incidence of direction selectivity was higher in VP (16/36) than in V2 (4/24) and the same was true for disparity (VP:21/36, V2:7/28). Interestingly, only two of the cells in V2 were selective for more than two of the parameters that we tested for, whereas nearly a third of the VP cells were selective for as many as three parameters (e.g., color, orientation and disparity). This may indicate an increase in the amount of qualitatively different visual information processed by individual cells at higher cortical levels.

These results allow for several interesting comparisons with previous studies of V2, V3 and V4 in dorsal extrastriate cortex. The high incidence of color-sensitivity in VP contrasts with a very low incidence reported for V3, providing further evidence that these are indeed distinct visual areas. There is also a difference in the incidence of color-sensitivity for ventral versus dorsal parts of V2, but it is smaller in magnitude and may reflect sampling biases or different criteria in classification. In any event, there clearly is more than one extrastriate area in the macaque that is involved in the analyses of color. V2 and VP appear to carry out other aspects of visual processing as well.

232.7 The Connections of the Middle Temporal Visual Area in the Macaque Monkey. J.H.R. Maunsell and D.C. Van Essen, Caltech, Pasadena, CA.

The middle temporal area (MT) in the macaque monkey is a small, but well-defined area in the superior temporal sulcus that is specialized for the analysis of visual motion. While MT has been shown to receive direct inputs from V1 and V2, little is known about its other connections in the macaque. We have made combined injections of 3H-proline and horseradish peroxidase (HRP) into MT to demonstrate its inputs and outputs.

Three hemispheres received injections which involved only cortex within the myeloarchitectonic borders of MT. The corpus callosum in each animal was cut prior to sacrifice, and the pattern of callosal inputs in the cortex was used as an aid in assigning connections to specific visual areas. The patterns of connections demonstrated were consistent among the different injections. Connections were found between MT and many other cortical areas, and most of these pathways were shown to be reciprocal. The laminar distributions of cells of origin (HRP) and sites of termination (3H-proline) were used as a basis for designating each labeled projection as either forward (ascending) or feedback. Pathways providing ascending input to MT were seen from V1, V2, V3, the ventral posterior area (VP), and a region anterior to VP in ventral extrastriate cortex. The major ascending outputs of MT were to two previously unidentified areas. One of these was the region of cortex immediately medial to MT in the superior temporal sulcus and extending a few millimeters onto the anterior bank of the sulcus. We have designated this MT-recipient cortex the medial superior temporal area (MST). The other output was to a small region, 3-5 mm in extent, near the fundus of the intra-parietal sulcus, which we have designated the ventral intra-parietal area (VIP). Connections with V4 also were demonstrated, but these were not clearly forward or feedback in nature.

Physiological recordings at or before the time of injection were used to determine precisely the visual field representation at each injection site. For all three injections, connections with cortical areas which have known topographic organization were between representations of the same part of the visual field. There was no obvious topographic organization of the outputs to MST and VIP, however.

Projections were also seen from MT to numerous sub-cortical structures. These included the inferior and lateral subdivisions of the pulvinar, the pontine nuclei, the pregeniculate nucleus, the claustrum, the reticular nucleus of the thalamus, the superior colliculus, the caudate nucleus, and the putamen. Of the sub-cortical projections, only those to the pulvinar were shown to be reciprocal. Supported by NIH Grant EY 02091.

232.8 DIRECTION AND ORIENTATION SELECTIVITY OF NEURONS IN AREA MT OF MACAQUES. T.D. Albright* and C.G. Gross. Dept. Psychology, Princeton Univ., Princeton, NJ 08544.

Visual area MT is a topographically organized prestriate area that lies along the posterior bank of the superior temporal sulcus. MT neurons are selective for direction of stimulus motion and this directional selectivity is organized in an orderly columnar fashion. Most macaque MT neurons were orginally reported to be insensitive to stimulus orientation. We now report a systematic study of the direction and orientation selectivity of MT neurons.

Animals were paralyzed and anesthetized with N_2O. Visual stimuli consisted of small spots and elongated slits of light, flashed within or moved through the receptive field. Among 44 MT neurons tested with a single small spot, 42 (95%) were selective for its direction of motion. The mean spot directional tuning bandwidth (BW: full width, half max.) was 108° with a range of 22-279°. The mean spot directional tuning index (DI: 1 - response in null direction/response in preferred direction) was 0.94 with a range of 0.41-1.11. All 53 cells tested with a moving slit were selective for its direction of motion. The mean slit BW was 85° with a range of 29-261°. The mean slit DI was 0.89 with a range of 0-1.82. Among 37 cells tested with both a moving slit and a moving spot, 36 (97%) were selective for both. However, the spot tuning was generally broader (mean BW difference = 15.0°.) while the DI's for the two groups did not differ.

Among 46 cells tested with a stationary flashed slit, 44 (96%) were selective for its orientation. However, the magnitude of the response to the optimum stationary slit was invariably smaller than that to the optimum moving spot or slit. Phasic on and off responses were generally seen. The mean orientation BW was 49° with a range of 9-133°. Finally, among 41 cells tested for both direction and orientation preference, 17 had a preferred axis of motion for a moving spot that was incongruent with the preferred orientation of a stationary slit (i.e., the two preferences were not at right angles). For these cells the preferred direction for the moving slit often fell in between the stationary orientation preference and the spot direction preference.

In summary, the majority of cells in MT were highly selective for the direction of motion of both a spot and an elongated slit. Tuning bandwidth was slightly narrower for the moving slit. In contrast to previous observations in macaques and in agreement with recent observations from owl monkeys, the large majority of MT neurons are also sensitive to the orientation of a stationary slit.

232.9 DIRECTION SELECTIVE RESPONSES TO SEQUENTIALLY FLASHED STIMULI IN EXTRASTRIATE AREA MT IN THE AWAKE MACAQUE MONKEY. W. T. Newsome, A. Mikami* and R. H. Wurtz. Laboratory of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20205.

In psychophysical experiments, human observers can perceive motion when viewing an appropriate sequence of flashed stimuli even though each individual stimulus is in fact stationary (apparent motion). MT, an extrastriate visual area which has been identified in several primate species, contains a high proportion of direction selective (DS) neurons and is thus thought to play a role in the processing of motion information. We have begun an investigation of the effects of sequentially flashed stimuli on MT neurons with the goal of defining the parameters of such sequences that are necessary to elicit DS responses.

Experiments were performed in an awake macaque monkey trained to fixate a small, stationary spot of light. Direction and velocity tuning for each MT neuron were determined using a computer controlled series of smoothly moving stimuli. Sequentially flashed stimuli (0.7° square) were then presented by projecting a stroboscope through a four-leaf diaphragm onto an x-y mirror galvanometer system which swept the image through the neuron's receptive field in the preferred direction and then in the null direction. The stroboscope flash duration was 10 μsec so that each flashed stimulus was essentially stationary (<.001° displacement) at the mirror velocities we used.

In general, low flash frequencies (interflash interval, IFI, >150 msec) elicited equivalent responses to sequences in both preferred and null directions: the discharge for each direction was a series of transient responses, one to each stroboscope flash. However, as the flash frequency was increased (at constant mirror velocity), a transition point was reached where neuronal discharge ceased for the null direction and the neuron became direction selective. For a mirror velocity of 15°/sec, this critical frequency ranged from 12 Hz (IFI = 83 msec, interflash distance, IFD, = 1.25°) to 18 Hz (IFI = 56 msec, IFD = .84°) for the neurons we studied. As mirror velocity was increased, a higher flash frequency was required to elicit DS responses from a given neuron. In other experiments the IFI and IFD were varied independently of each other. At appropriate values of IFD, DS responses could generally be elicited for IFI's up to 70 msec, and occasionally up to 200 msec. At appropriate IFI's, DS responses were generally obtained for IFD's as large as 1.5°. Various optimal values of the IFI for the perception of apparent motion have been reported in the psychophysical literature: 20-30 msec (Sperling, 1976), up to 70 msec (Morgan and Turnbull, 1979), up to 40 msec (Morgan, 1980). Values of IFI which are optimal for the perception of apparent motion in humans seem to be a subset of those which will elicit DS responses in MT neurons in monkeys.

232.10 SUBDIVISIONS AND CONNECTIONS OF INFERIOR TEMPORAL CORTEX IN OWL MONKEYS. R. E. Weller and J.H. Kaas. Departments of Psychology and Anatomy, Vanderbilt University, Nashville, TN 37240.

We investigated inferior temporal cortex (IT) of owl monkeys (Aotus trivirgatus) because the organization of IT cortex is poorly understood in New World primates, and because much of IT's cortical input seemed likely to come from subdivisions of extrastriate cortex that have been well-defined by microelectrode mapping methods in owl monkeys (Allman and Kaas, Science, 191: 572,1976). Single injections of ^{3}H-proline and/or horseradish peroxidase were placed in different parts of IT of 11 owl monkeys, and in subdivisions of cortex which were found to have connections with IT in 13 other owl monkeys. Patterns of connections were subsequently related to the architectonically defined borders of various cortical areas. The patterns of connections and the cortical architecture strongly suggest that the IT region, including the ventral surface of the temporal lobe, contains at least three and possibly four subdivisions.

The caudal half of IT is distinguished by reciprocal ipsilateral connections with the Dorsolateral Visual Area (DL) and a region of cortex immediately dorsomedial to DL. The projections to IT from DL and cortex dorsomedial to DL are bilateral. There appears to be no input to IT from V I, V II, the Middle Temporal Visual Area, the Medial Visual Area, and the Posterior Parietal Area, and there is little or no input from the Dorsomedial Visual Area. This receiving zone of IT was subdivided into dorsal and ventral parts, IT_D and IT_V, because single injections in DL sometimes produced separate foci of label in these two parts, and because the two divisions were interconnected.

The rostral portion of the inferior temporal lobe, IT_R, receives input from IT_D and IT_V, but not from DL. Cortex on the ventromedial surface of the temporal lobe, IT_M, also receives input from IT_D and IT_V, but it differs from IT_R by receiving input from visual cortex rostral to MT.

The interhemispheric projections of IT are via both the anterior commissure and a caudal part of the corpus callosum. Subcortically, IT is strongly connected with subdivisions of the pulvinar complex, including a part of the superior pulvinar not previously shown to be related to visual cortex.

Architectonically, IT_D, IT_V, and IT_R look like Area TE of von Bonin and Bailey (1947), although regional variations are also present. IT_M closely resembles Area TF of von Bonin and Bailey (1947).

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232.11 VISUAL RESPONSES OF INFERIOR TEMPORAL NEURONS ARE MODIFIED BY ATTENTION TO DIFFERENT STIMULUS DIMENSIONS. Barry J. Richmond, and Takayuki Sato*, Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, MD. 20205.

Removal of the anterior part of inferior temporal cortex, area TE of Bonin and Bailey, produces severe pattern recognition losses. Given this result, our previous single unit recording studies in area TE yielded some unexpected findings. In awake monkeys trained to fixate a spot of light and to respond to its dimming (fixation task), the presence of the fixation spot consistently reduced neuronal responses to a receptive field test stimulus far below those obtained when the fixation spot was turned off before the test stimulus was presented (blink task). Furthermore, neuronal responses were again consistently reduced below those in the blink task when the monkey was required to make a behavioral response to the dimming of the test stimulus (blink + stimulus attention task). Finally, when both suppressive influences were present, neuronal response were weakest of all (fixation + stimulus attention task).

To determine whether the same results would be repeated when the animal was required to attend to the pattern of the test stimulus rather than just to its dimming, we compared responses of neurons in the foregoing tasks with their responses while the monkey was performing a pattern discrimination task in which the positive and negative stimuli were presented successively. In all tasks the retinal locus of the stimulus was closely controlled by the enforced requirement that the monkey maintain a constant eye position. In the discrimination task the fixation spot was present and the receptive field test stimulus was used as the negative discriminandum so that stimulus conditions on negative trials were identical to those in the original tasks. Also, both the fixation + stimulus attention task and the discrimination task required the monkeys to attend to the dimming of the test stimulus. Only in the discrimination task, however, was the monkey required to recognize the pattern of the stimulus. Under the latter condition a class of cells was found in which the strength of the response to the test stimulus exceeded that in the fixation + stimulus attention task, approaching or surpassing that seen when neither suppressive condition was present (i.e. the blink task).

The results indicate that in area TE the neuronal response to a visual stimulus depends not only on whether attention is directed to the stimulus but also on which particular stimulus characteristic is being attended to.

232.12 CHRONIC BLINDNESS FOLLOWING NONVISUAL LESIONS IN MONKEYS: PARTIAL LESIONS AND DISCONNECTION EFFECTS. Richard K. Nakamura and Mortimer Mishkin, NIMH, Building 9 Room 1N107, Bethesda, MD 20205.

We have previously reported that chronic blindness can be produced in monkeys by a large cortical removal that preserves the modality-specific visual cortex (striate, prestriate, and inferior temporal) but includes all other cortical areas (Neurosci. Abs. 5:800, 1979). The lesion was placed in only one hemisphere, the other hemisphere having been left intact but visually deafferented by optic tract section and forebrain commissurotomy. We subsequently showed with unit recording that normal processing of visual information continued in the retinally connected visual cortex despite absence of any behavioral signs of vision (Neurosci. Abs. 6:578, 1980). We now report on further attempts to analyze the reasons for the blindness.

The large nonvisual cortical lesion can be viewed as being composed of three major components: sensorimotor, limbic, and polysensory regions. Effects on vision of removing individual components as well as all two-component combinations indicate that the three regions are graded in importance, with polysensory cortex most and sensorimotor cortex least important; all components, however, make some contribution since some visual recovery is possible if any is preserved.

In another study, we found that animals prepared with a unilateral optic tract cut and a contralateral nonvisual lesion but with the forebrain commissures intact recovered vision within an average of 10 days of surgery. This recovery included accurate visually guided reaching as well as ability to perform a pattern discrimination task. Subsequent forebrain commissurotomy, however, rendered them permanently blind.

Taken together, these data suggest that the chronic blindness is the result of a disconnection of the modality-specific visual processing areas from further stages of processing, with polysensory cortex providing the most important stage.

232.13 COLOR-GENERATING INTERACTIONS ACROSS THE CORPUS CALLOSUM. Edwin H. Land*, David H. Hubel, Margaret S. Livingstone, S. H. Perry* and Michael M. Burns* (SPON: D. Potter). Rowland Inst., Dept. Animal Neurobiology, Harvard Medical School and Polaroid Corporation, Cambridge and Boston, MA 02139.

Few would disagree that synthesis of information over large parts of the visual field must happen either in the retina or in the brain, or both. Computations for color clearly occur across large regions of the visual field (Land,1964) with little or no interocular transfer. The calculation must occur at some level in the visual pathway receiving information from a wide area of the visual field yet keeping the information from the two eyes effectively separate. One cannot offhand rule out the retina since, for movement, wide lateral interactions are known to occur (McIllwain '64).

To settle this question we asked whether the calculation could occur across the vertical midline of the visual field of a man whose corpus callosum had been surgically severed. We arranged a display in which the point of fixation was surrounded by an area subtending 6° illuminated throughout the experiment by a constant flux. This area was seen by normal observers as either white or purple depending upon how a large Mondrian, confined to one half of the visual field, was lit by three illuminators projecting short, medium, and long visible wavelengths. The two colors, although identical in flux to the eye, were clearly distinguishable and identified by normal observers as "white" and "purple".

We required our subject, kindly referred to us by Dr. A. Reeves, to fixate on the center of the test area by having him read aloud bilaterally symmetrical letters that appeared one after another in a small window. We instructed him to keep reading the letters out loud to ensure fixation, and whenever asked, after each change of illumination of the Mondrian, to name whatever color he saw in the area around the window. When the Mondrian was in the left visual field the subject called the test area arbitrarily white or purple without any relationship either to the illumination of the Mondrian or to the color for normal observers. The computation presumably failed because the Mondrian and the part of the test area reported on by the verbal left hemisphere lay in different half-fields and so required the corpus callosum, which had been severed. Contrariwise, when the Mondrian was in the right visual half-field (which could be accomplished by viewing it with a mirror) the subject's description of the color clearly agreed with the appearance to normal observers and was determined predictably by the illumination of the Mondrian. In this situation the test area reported on lay in the same visual half-field as the Mondrian and its color was therefore dependent on the Mondrian, as in normal subjects. The experiment as a whole indicates that the color computational system is cortical and not retinal.

233.1 NUMBER OF AXONS IN THE OPTIC NERVE OF THE DEVELOPING RHESUS MONKEY: OVERPRODUCTION AND ELIMINATION BEFORE BIRTH.
P. Rakic and K.P. Riley*, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

The number of optic axons was determined by electron-microscopy in eleven rhesus monkeys ranging in age from the 47th embryonic day (E47) to adulthood. (Birth in this primate occurs around E165). All animals were fixed by vascular perfusion with mixed aldehydes. In each case, a segment of the optic nerve was dissected midway between the eyeball and chiasm and processed for electron-microscopic analysis. All axons, growth cone profiles and nonneuronal elements were measured and counted in each of an uninterupted series of photomontages prepared along two diagonal radii of the optic nerve from overlapping electronmicrographs at X20.000. The total number of optic fibers for each age was estimated from the total cross-sectional surface area of the optic nerve, the area occupied by axons in the nerve and their relative density. In agreement with previous studies, the optic nerve in the adult monkey contains between 1.2 and 1.3 x 10^6 axons. At E47 all retinal axons are unmylineated, relatively uniform in diameter, and their number is about 0.7 x 10^6, or slightly more than half of the number counted in adults. During the next three weeks the number of axons increases rapidly reaching 2 x 10^6 at E54 and over 2.85x 10^6 by E69. This number which is more than double the adult value, is maintained for the next three weeks according to measurements in E83 and E95 specimens. Thereafter, axonal growth cones disappear from the optic nerve the size of axonal diameters becomes highly variable, and the largest axons acquire myelin sheaths. Most importantly, the number of optic fibers decreases dramatically to 1.9 x 10^6 at E110 and to about 1.7 and 1.8 x 10^6 in two fetuses sacrificed at E135 and E149, respectively. After birth, the number of axons continues to decline at a slow rate approaches adult values only after the second postnatal month. The sharpest reduction, a loss of over one million axons, is synchronized with the emergence of six cellular laminae in the lateral geniculate nucleus and with the segregation of retinal terminals which initially overlap in this nucleus (Rakic, P.'76, Nature 261: 467). The process of elimination of retinal axons may depend on competition within the lateral geniculate nucleus since monocular enucleation at or before the stage of axonal reduction prevents the segregation of retinal input (Rakic, P.'81, Science 214: 928). It may be significant that in the rhesus monkey retinal axons are both produced in excess and pruned in number mainly before birth and hence prior to visual experience. Supported by EY-02593.

233.2 THE DEVELOPMENT OF LAMINAR PATTERNS OF EXTRARETINAL PROJECTIONS IN THE DORSAL LATERAL GENICULATE NUCLEUS.
J. K. Brunso-Bechtold, S. L. Florence* and V. A. Casagrande, Dept. of Anatomy, Vanderbilt University, Nashville, TN 37232

The dorsal lateral geniculate nucleus (LGN) in the mature tree shrew has 6 layers which can be defined by cytoarchitectural features, i.e. characteristic interrelationships of cell groups, such as interlaminar spaces (ILS); cytological features, i.e., characteristics of cells themselves such as cell size; or pattern of termination of afferents. Of the latter, one of the ways the retinal afferents define layers is by concentrating almost exclusively over cell layers while extraretinal afferents concentrate within ILS. For example, afferents from the superior colliculus concentrate in the more lateral ILS while afferents from striate cortex terminate equally heavily within all ILS. The time course of development of these extraretinal afferents is of interest since they establish a laminar pattern of terminations well after the retinal afferents. The retinal terminals have established a laminar pattern by birth, prior to the cytoarchitectural and cytological definition of layers. The extraretinal afferents however are not laminated at birth. For example, the corticogeniculate fibers have only reached the medial-most layers of LGN. The collicular fibers are present throughout the mediolateral extent of the nucleus but do not show a concentration in the future ILS. During the first postnatal week, the corticogeniculate fibers enter the nucleus and begin to show a concentration in the ILS as these spaces begin to appear. Interestingly, although the collicular fibers are present prior to the cortical fibers, they appear to lag behind the corticogeniculate fibers in showing the mature pattern of termination in the ILS. These results suggest that the timing of the arrival of various geniculate afferents may play a role in ILS formation. Furthermore in the absence of retinal afferents, and consequently ILS (Brunso-Bechtold & Casagrande, 1981), corticogeniculate fibers do not terminate at the laminar borders but instead spread topographically throughout the LGN layers. In that case, the late arriving corticogeniculate fibers may terminate in place of the absent retinogeniculate terminals presumably more proximal to the cell body. This shift in the pattern of termination of corticogeniculate fibers may, in fact, play a role in the failure of ILS formation in bilaterally enucleated animals. (Supported by EY01778 (VAC); KO4-EY00223 (VAC); EY03881 (JKB-B & VAC)).

233.3 THE STRUCTURE AND DEVELOPMENT OF THE DORSAL LATERAL GENICULATE NUCLEUS AND ITS RETINAL AFFERENTS IN THE ALBINO FERRET (MUSTELA PUTORIUS FURO). Josephine Cucchiaro and R.W. Guillery. Comm. Neurobiology and Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

The development of the abnormal retinogeniculate pathway and of the characteristic abnormal pattern of cellular lamination in the dorsal lateral geniculate nucleus (dLGN) of albino ferrets has been studied. In order to define the first appearance of the abnormality, 62 albino ferrets (age E32 through adult) and 18 pigmented ferrets (age-matched to albino littermates) were given monocular injections of either tritiated proline or horseradish peroxidase. Survival times of 18-24 hours were used for postnatal animals, 8 hours for fetuses injected in utero. These times were sufficient for the transport of tracer to the superior colliculus. Sections were processed for autoradiography or with TMB.

The adult albino has an unusually small ipsilateral retinogeniculate projection, which terminates in abnormal parts of the nucleus and is divided into two separate patches which innervate the extreme ends of lamina A1. Intermediate portions of A1 receive an abnormal crossed innervation from the contralateral eye. This abnormal pattern of retinal innervation is associated with an abnormal pattern of cellular lamination in the dLGN.

Retinal afferents arrive in the dLGN at about the same time in albino and pigmented animals. On day E32 contralateral fibers first begin to invade the dLGN. Ipsilateral fibers are in the optic tract at this time but are not in the nucleus until E34. In albinos on E32 the ipsilateral projection in the optic tract is sparser than normal and occupies only the anterior portion of the zone normally occupied by ipsilateral fibers. At birth, ipsilateral and contralateral projections extend throughout the dLGN, however in albinos the ipsilateral projection is much sparser than normal. On postnatal day 4 in the albino the ipsilateral terminals become localized to an abnormal part of the dLGN, lying close to the optic tract and extending into the region that in normal animals forms the monocular segment. By P8 the ipsilateral terminals have subdivided into two separate patches. Not until P12 can any hint of the albino pattern of cellular lamination be detected, and by P17 the lamination pattern is like that seen in the adult.

These results show that in the albino ferret the ipsilateral fibers are reduced in number and are abnormally distributed in the optic tract before their invasion of dLGN. The time of arrival of retinal afferents at the dLGN is normal and initially the ipsilateral projection extends to all parts of the nucleus, as in the normal animal. However, in the albino the ipsilateral projection is sparser at all ages and becomes abnormally distributed within the nucleus well before the appearance of the abnormal pattern of cellular lamination. Thus, in the albino ferret the ipsilateral fiber abnormality precedes the arrival of the ipsilateral fibers in the dLGN and the appearance of abnormal cellular lamination.
(Supported by NIH grants 5RO1 EY-02374 and 5T32 GM-07839.)

233.4 THE EFFECTS OF BINOCULAR ENUCLEATION ON THE DEVELOPMENT OF THE DORSAL LATERAL GENICULATE NUCLEUS (DLGN) OF THE FERRET. A-S. LaMantia* and R.W. Guillery (SPON: E.H. Polley). Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

In the ferret, retinal afferents first reach the dLGN on the 32nd day of gestation (E32) (Cucchiaro & Guillery, this volume); they begin to segregate into ipsilateral and contralateral terminal fields between post-natal days (PND) 2 and 7, and cellular laminae begin developing in the dLGN thereafter (Linden et al., J. Comp. Neurol., 203:189-211, 1981). We have compared the effects of binocular enucleation on E29 with the effects produced by later enucleations, at birth (PND 0) and on PND 7. Binocular enucleations at any time between E29 and PND 7 produce identical sets of abnormalities which begin to appear between PND 8-12 and become more severe subsequently. Abnormalities are less dramatic when enucleations are done later than PND 7.

Binocular enucleations between E29 and PND 7 produce the following abnormalities: interlaminar zones fail to appear at any time and layers A and A_1 cannot be distinguished from each other; the dLGN fails to develop its normal "L" shape and its borders become gradually less clear; the cell free spaces separating the dLGN from the perigeniculate nucleus and from the medial interlaminar nucleus are gradually obscured and eventually neither nucleus can be easily distinguished from the dLGN; dLGN neurons of the adult are noticeably smaller and cell density appears decreased, suggesting a loss of cells which is currently being studied. When retinal afferents are removed after PND 7 at progressively later stages, each of these abnormalities appears diminished, and the normal cytoarchitectonic characteristics of the dLGN survive for a longer period. These results suggest that some geniculate lamination (ipsi/contra) is dependent upon the presence of retinal afferents after PND 7. However, other geniculate features are not affected by removal of both eyes. The distinction between the A (A and A_1) and magnocellular C layers and the parvocellular C layers does develop regardless of age at time of enucleation. The characteristic radial arrangement of the neurons of the A layers along the lines of projection is somewhat obscured, but the characteristic relative size, location and orientation of the parvocellular C neurons (small elongate neurons at the lateral border of the dLGN, with their long axes parallel to the optic tract) is quite apparent. Therefore, the cellular lamination that corresponds in the normal adult to the differential distribution of functionally distinct retinogeniculate afferents (X, Y and W) is the result of a developmental process intrinsic to the dLGN, perhaps dependent upon events occurring at the time of dLGN cell production and migration (Hickey and Cox, Soc. Neurosci. Abstr., 5:788, 1979; Shatz, Soc. Neurosci. Abstr., 7:140, 1981) but is not influenced by the presence of retinal afferents.
(Supported by NIH Grant 5RO1 EY-02374.)

233.5 GENESIS OF DIFFERENT RETINAL GANGLION CELL TYPES IN THE CAT. M. Kliot and C.J. Shatz. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

In the retina of the adult cat at least three different classes of ganglion cells can be distinguished anatomically. To find out when during development these different classes of neurons are generated we made intrauterine injections of ^{3}H-Thymidine (Hickey and Cox, Neurosci., 5:788, 1979) into 8 fetuses of known gestational age (gestation: 65 days) and then perfused the animals at 5-9 weeks postnatally. Retinae were flat-embedded in methylmethacrylate and 5µm sections were cut parallel to the ganglion cell layer and processed for autoradiography.

Injections on embryonic day 19 (E19) did not label any cells in the retina. At E21 medium and small ganglion cells were labeled primarily in and around the area centralis. Injections on E24 and E26 labeled prgressively more of the medium and small ganglion cells as well as a few of the large cells. Again labeled neurons were most heavily concentrated in and around the area centralis, but also extended further peripherally, almost reaching the ora serrata by E26. An injection on E27 labeled ganglion cells of all three size classes throughout the retina. By E30 primarily small and large cells were labeled everywhere. At later ages (E35,E39, E46,E56) only a few very small cells were labeled. Their time of genesis coincided with that of cells situated along the vitreal margin of the inner nuclear layer, suggesting that these cells might be displaced amacrine cells. These findings show that extensive overlap, both temporal and spatial, occurs in the genesis of the different classes of ganglion cells (cf. Polley et al. Neurosci., 7:672, 1981).

In order to determine how the adult distribution of labeled cells arises, 8 fetuses were injected at particular embryonic ages and then sacrificed at varying times thereafter. Fetuses injected on E24 and sacrificed at E30 or E34 showed a nonuniform distribution of heavily labeled cells within the retina, with fewer present peripherally. Another fetus was injected on E26 and survived to E40. Numerous heavily labeled cells within the ganglion cell layer, primarily its vitreal half, were distributed roughly uniformly throughout the retina. In a fetus injected on E26 but sacrificed at E54, a center-to-peripheral gradient of decreasing density of labeled cells was seen, but it was not as sharp as that found in the adult. This change in the distribution of labeled cells between E40 and E54 parallels the change in the overall distribution of cells within the ganglion cell layer found by Stone et al. (Devel. Brain Res., 2:231-242, 1981) suggesting that the final distribution of retinal ganglion cells is not dependent on birthdate alone.

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233.6 FUNCTIONAL RETINOGENICULATE SYNAPSES IN FETAL CATS. C.J. Shatz, P.A. Kirkwood* and M.W. Siegel. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Prenatal development of the cat's retinogeniculate pathway is characterized by an initial period in which afferents from the two eyes are intermixed within the lateral geniculate nucleus (LGN), followed by segregation of the afferents into their familiar laminar pattern by birth (Shatz & DiBerardino, Soc. Neurosci., 1980). We wished to determine whether functioning synaptic connections between retina and LGN exist during these times.

Electrical stimuli were delivered via suction electrodes to the optic nerves or tracts of isolated diencephalon preparations made from animals of known gestational age between embryonic day 42 (E42) and postnatal day 2 (P2) and maintained in vitro. Extracellular microelectrode recordings were made from single units within the LGN. At all ages, both pre- and postsynaptic single-unit activity could be recorded and clearly distinguished. So far, a total of 62 postsynaptic units have been studied in 10 fetuses. When tested with double shocks, many of the units responded more strongly or at lower threshold. Unit responses in the younger fetuses were typified by high thresholds, variability, and fatigability. With increasing age, more units were encountered with precise thresholds and secure firing. At all ages tested so far (E43 through P2) it was possible to find some postsynaptic units that received dual input from stimulation of both optic nerves. At P2, units were also found in which the response to stimulation of one nerve was inhibited by stimulation of the other.

These findings were supported by results from other experiments in which retinogeniculate synapses were identified ultrastructurally following the anterograde axonal transport of horseradish peroxidase (HRP) injected intraocularly in 17 fetuses aged E46 to P2. At all ages, synapses were found in which the presynaptic profile was filled with HRP reaction product. Synapses were also seen within the LGNs of fetuses as young as E40, but as yet the presynaptic elements have not been conclusively identified.

These physiological and morphological observations indicate that functioning retinogeniculate connections may exist in vivo as early as E42 in the cat: roughly one week before the onset of segregation. Further, prior to the completion of segregation, at least some LGN neurons receive binocular excitation. We conclude therefore that here, as in other areas of mammalian nervous system including the primary visual cortex, the adult pattern of retinogeniculate connections is achieved in part by elimination of already functional synapses.

Supported by grants from N.I.H. (EY 02858 and BRSG RR5353) and the McKnight Foundation to C.J.S. P.A.K. is an M.R.C. Travelling Fellow on leave from the Institute of Neurology, London.

233.7 NEUROGENESIS OF THE CAT'S LATERAL GENICULATE NUCLEUS: A ^{3}H-THYMIDINE STUDY T. L. Hickey and Peter F. Hitchcock*. School of Optometry/The Medical Center, University of Alabama in Birmingham, Birmingham, AL 35294

Neurons that will ultimately form the dorsal (DGL) and ventral lateral geniculate nuclei, the medial interlaminar nucleus, the perigeniculate nucleus, and the nucleus reticularis undergo their final cell division; i.e., cell-birth, beginning on or slightly before embryonic day 22 (E22) and ending on or before E32. Early in this period neurogenesis occurs for all of these regions, while only DGL cell-birth continues until E32. Although the thalamus as a whole exhibits fairly distinct ventral-to-dorsal and lateral-to-medial spatiotemporal gradients of cell-birth, such gradients are not as obvious within any of the nuclear groups studied. For example, DGL neurons born on any given day during much of this period can be found distributed throughout the nucleus in the adult cat. Furthermore, DGL neurons born during the first 7 days (E22-E28) of this 11 day period exhibit a full range of soma sizes, while DGL cell-birth during the last 4 days of this period (E29-E32) is limited to neurons with smaller somas.

To at least some degree the location and size of a DGL cell provide clues as to that cell's functional properties. Based on presently available information regarding the relationship between soma size (large vs. small), location (A laminae vs. C laminae) and physiology (X, Y, or W), the findings described above suggest that DGL neurons of all functional classes are born simultaneously throughout most of this 11 day period.

On the other hand, a much more accurate prediction of functional properties can be made for DGL cells if other morphological characteristics such as the orientation, laminar distribution and morphology of the cell's dendrites are also taken into account (Friedlander, Lin, Stanford, and Sherman, J. Neurophysiol., 46, pp. 80-129, 1981). With this thought in mind, and in an effort to provide additional support for our interpretation of the above findings, we developed a technique that allows us to combine Golgi impregnation procedures and ^{3}H-thymidine autoradiographic techniques. The combination of these two techniques allows us to define birthdates for DGL neurons with known morphological characteristics (Guillery, J. Comp. Neurol., 128, pp. 21-50, 1966). So far the results of this latter study show that geniculate cells of all morphological classes can undergo their final cell division on the same day, a finding that fits quite well with our previous results.

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233.8 CATECHOLAMINES CAN INFLUENCE THE SEGREGATION OF RETINOGENICULATE PROJECTIONS IN RATS. L.L. Rose* and P.W. Land. Department of Anatomy, M.U.S.C., Charleston, S.C. 29425.

We have begun examining the effects of altered catcholamine levels on the development of retinal projections in Long-Evans black-hooded rats. Litters of newborn rats were divided into three groups: control; 6-hydroxydopamine (6-OHDA) treated; norepinephrine (NE) treated. We administered 6-OHDA (SIGMA; 100mg/kg free base in 0.5% ascorbate) subcutaneously on days 0, 1, 2 and 3. This procedure specifically destroys central noradrenergic fibers, leaving dopaminergic fibers intact. NE treated littermates received a total of 100mg/kg NE (- arterenol; SIGMA) in 0.5% ascorbate in one or two subcutaneous injections on each of days 0, 1, 2, 3, and 4 after birth. NE treated animals also received the MAO inhibitor iprianozid phosphate (100mg/kg in 0.9% saline) 20-30 minutes prior to each NE injection. Control animals received injections of ascorbate or were uninjected.

In order to test the influence of the above procedures on the development of retinal projections, we injected one eye of animals from each group with 30% HRP in 2% DMSO on day 6, 7, 8, 9, or 10. Animals were sacrificed 18-24 hrs. later; frozen sections through their brains were cut at 40µm and reacted with tetramethyl benzidine.

In normal pigmented rats the projections from the two retinae initially overlap in the lateral geniculate nuclei (LGN). By around day 5, axons from the ipsilateral and contralateral retinae begin to segregate, and by day 10 they occupy mutually exclusive regions of the LGN. This sequence was confirmed in our control animals. 6-OHDA and NE treatment altered this normal pattern. In both cases, crossed retinogeniculate axons were slower to withdraw from the region occupied by uncrossed axons, and at 10 days postnatal there still was considerable overlap in the projections from the two retinae. Moreover, while in 6-OHDA treated animals the uncrossed projection seemed to be of normal density and distribution at 10 days, uncrossed axons in NE treated animals were much more broadly distributed in the LGN. Thus, both depletion and brief augmentation of normal NE levels dramatically affect the outcome of interaxonal interaction in the developing visual system. These results reaffirm the notion that the monoaminergic fiber systems play a significant role in the early shaping of the vertebrate brain. (Supported by NIH grant EY03447).

233.9 BINOCULAR LID SUTURE CAUSES THE DEVELOPMENT OF ABNORMAL STRUCTURE/FUNCTION RELATIONSHIPS AMONG LATERAL GENICULATE NEURONS IN THE CAT. D. Raczkowski, L.R. Stanford and S.M. Sherman. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Cats raised with binocular lid suture fail to develop the normal complement of Y-cells in the dorsal lateral geniculate nucleus (LGN) despite the normal growth of geniculate somata. In order to determine more precisely the relationship between physiology and anatomy for these cells, we employed techniques previously applied to normal[1] and monocularly lid sutured[2] cats. That is, we used micropipettes filled with horseradish peroxidase (HRP) to record from a single LGN neuron, physiologically classify it, and intracellularly fill it with HRP for subsequent morphological analysis of its soma, dendrites and axon. X- and Y-cells were identified by a standard battery of tests (response latency to optic chiasm shock, linearity of spatial summation, etc.), and abnormal cells that responded poorly or irregularly were also recorded. Eighteen neurons have been recovered from the A-laminae of cats reared to adulthood with binocular lid suture and the following comparisons with LGN cells in normal cats were made. 1) Some cells had normal physiology and morphology. 2) Several neurons had normal Y-cell responses to electrical and visual stimulation; their morphological features were characteristic of normal Y-cells except for abnormally small somata. 3) Several neurons displayed poor and irregular responses that, when present, were similar to those of Y-cells; these neurons exhibited abnormal morphology, including tortuous, thin and beaded dendrites. 4) Some cells had normal X-cell physiology and displayed morphology characteristic of normal Y-cells. 5) Finally, two cells were visually unresponsive; one had the morphology of a normal Y-cell and the other, of a normal X-cell. These abnormalities in structure/function relationships are qualitatively similar to those seen in deprived laminae of monocularly sutured cats and suggest deficits in the Y-cell pathway that are more pronounced than those in the X-cell pathway. However, more data are needed to establish both the relative degree to which X- and Y-cell development is affected by binocular deprivation and the extent to which monocular and binocular suture cause the same abnormalities in structure/function relationships.

Supported by USPHS Grant EY03038.

1. Friedlander, M.J., C.-S. Lin, L.R. Stanford and S.M. Sherman (1981), J. Neurophysiology, 46:80-129.

2. Friedlander, M.J., L.R. Stanford and S.M. Sherman (1982), J. Neuroscience, 2:321-330.

233.10 EFFECT ON SEGREGATION OF RETINOGENICULATE PROJECTIONS IN EARLY UNILATERAL ENUCLEATED OR DARKED-REARED HAMSTERS. K.-F. So, H.H. Woo* and L.S. Jen. Dept. of Anatomy, Univ. of Hong Kong, Hong Kong.

In adult golden hamsters, the dorsal lateral geniculate nucleus (LGd), which lacks a noticeable pattern of cellular lamination, receives predominantly contralateral retinal input except a medial segment where the ipsilateral fibers from the other eye terminate. The development of the retinogeniculate projections from both eyes in the hamster goes through a process of initial overlap and subsequent segregation. By day 8, adult-like bilaminar segregation of contralateral and ipsilateral inputs is achieved (So et al, Brain Res., 142:343, 1978).

In order to investigate if axo-axonal interaction is important for the progressive segregation phenomenon, one eye of 19 hamsters was removed on day zero and the projections from the remaining eye were studied on various postnatal ages (day 2 to 17) with the anterograde horseradish peroxidase (HRP) method using tetramethyl benzidene as chromogen. In all stages of development, retinogeniculate fibers from the remaining eye were observed to distribute evenly throughout the entire contralateral nucleus including the medial segment which normally receives ipsilaterl retinogeniculate projections. The ipsilateral retinogeniculate projections, unlike that in the normal animals, still occupied a large area of the LGd on and beyound day 8. These results suggest that binocular competition is important for the segregation phenomenon.

This binocular competition does not seem to depend on patterned visual experience because the segregation phenomenon is close to completion on day 8 but the eyes of the hamsters are not open until day 15. However, we cannot rule out the possibility that diffuse light stimulation may play a role in the segregation phenomenon. In order to investigate this possibility, three pregnant mothers were allowed to give birth in the dark and a total of 10 babies were reared in the dark until day 8, 10 or 12 when their retinogeniculate projections were studied with the anterograde HRP method. The results showed that in all the animals studied, the fibers from both eyes segregated into the adult-like bilaminar pattern. This suggests that diffuse light stimulation is not important in mediating the binocular competition which underlies the segregation phenomenon. Whether spontaneous activity of retinal ganglion cells is important for this process remains to be investigated.

(Supported by Research Grants from University of Hong Kong and Wing Lung Bank Medical Research Fund).

233.11 **SEGREGATION OF RETINOGENICULATE PROJECTIONS IN HAMSTERS WITH EARLY UNILATERAL VISUAL CORTICAL REMOVAL.** **L.S. Jen and K.-F. So. Dept. of Anatomy, Univ. of Hong Kong, Hong Kong.**

In adult golden hamsters, the majority of the retinogeniculate fibers are crossed and project to the entire dorsal lateral geniculate nucleus (LGd) except the dorsal medial region which is occupied almost exclusively by the ipsilateral projections. However, in the developing animals, the retinogeniculate fibers from both eyes overlap with each other and subsequently segregate to form the adult-like bilaminar pattern on day 8 (So et al, Brain Res. 142:343, 1978). If one eye is removed at birth, the bilaminar pattern is not observed at any stage of development suggesting that interaction between optic fibers arising from both eyes is important in determining the final pattern of terminal distribution of the retinogeniculate projections (So et al, this volume). Since it is known from our preliminary experiments that visual cortical fibers are in the process of growing into the geniculate nucleus on day 5, we would like to find out whether removal of these fibers has any effect upon the segregation phenomenon described above.

The left posterior cortex was removed in a series of day 5 old hamsters, followed by injection of the right or left eye with a 60 to 80% horseradish peroxidase solution on day 8,10 and 12. The animals were sacrificed 14 to 18 hours after the eye injection. The brains were frozen sectioned and processed according to the Mesulum method. The results indicated that segregation of the retinogeniculate fibers could be observed in all the animals studied despite of the fact that the size of the LGd ipsilateral to the cortical removal was greatly reduced due to retrograde degeneration. Thus, it seems that the segregation phenomenon of the retinal fibers is dependent on specific interaction of axons arising from both eyes. The fibers from the visual cortex, even though being a major input to the dorsal lateral geniculate nucleus, does not seem to play any significant role on the segregation phenomenon.

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233.12 CLOSE CORRELATION OF RETINOGENICULATE EXCITABILITY WITH TRANSNEURONAL ATROPHY IN THE PARTIALLY DEAFFERENTED LATERAL GENICULATE NUCLEUS. U.Th. Eysel, U. Mayer* and U. Wolfhard*. Institute of Physiology, University of Essen, D-4300 Essen 1, West Germany.

The number of remaining retinogeniculate inputs after partial deafferentation was discussed as possible determining factor of transneuronal atrophy in the monkey lateral geniculate nucleus (LGN) following monocular enucleation (Ghetti et al., Brain Research, 45:31, 1972). Observations in the partially deafferented LGN of the cat support this suggestion.

Nasal retinal lesions lead to deafferentation of the lateral parts of layers A, C and C2 in the contralateral LGN. In the present experiments microelectrode recordings were made in the border regions of normal innervation and visual deafferentation in layer A of the LGN more than 30 days after photocoagulation of the contralateral nasal retina. A gradual decrease of retinogeniculate excitability was observed. The LGN cells changed from normal excitability to total visual deafferentation over a medio-lateral distance of 250µm.

The border of the normally innervated region in the LGN was autoradiographically determined after injection of ^{3}H-proline into the lesioned eye. The boundaries of the regions of normal light-excitability and autoradiographic label corresponded to each other.

In series of frontal LGN sections (6 µm), counterstained with cresyl violet, cell size measurements were made in layer A with an image analyzer. A sequence of adjacent fields (100 µm width) was evaluated beginning 500µm inside the labelled region and progressing 600µm laterally into the unlabelled zone. In the labelled region the mean perikaryal cell areae were 225 to 275µm^2. Lateral to the border of the labelled region the cell sizes decreased gradually over a distance of 200 to 300µm. Further lateral the cells displayed mean perikaryal areae of 150 to 175µm^2.

The morphometrical and neurophysiological results show a parallel decay of cell size and retinogeniculate excitability. This indicates a close correlation of functional connectivity with transneuronal atrophy in the LGN of the adult cat. The amount of remaining retinogeniculate coupling after partial visual deafferentation seems to determine the degree of protection against transneuronal atrophy.

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234.1 EVIDENCE FOR THREE KINETICALLY DIFFERENT FORMS OF TYROSINE HYDROXYLASE IN RAT STRIATUM USING BH_4. L. Miller* and W. Lovenberg*. Sect. on Biochem. Pharmacol., NHLBI, NIH, Bethesda, MD 20205

Since the initial observation that tyrosine hydroxylase (EC 1.14.15.2; tyrosine 3 monooxygenase) (TH) is the rate limiting enzyme in catecholamine biosynthesis there have been numerous investigations in an attempt to uncover the regulatory features which underlie the limitation of synthesis rates in vivo. Besides end-product inhibition by catecholamines activation of the enzyme by phosphorylation has been suggested by many investigators to be one of the regulatory mechanisms. Both of these features have in common the interaction between enzyme and cofactor. The present investigation was undertaken to pursue this interaction using the natural cofactor, (6R) L-erythrotetrahydrobiopterin. To date no investigator has used solely the 6R form of BH_4 to examine TH in striatum. Rat striata were homogenized in 4 volumes of 0.5M KP, pH 7.2 containing 0.2% Triton X-100, centrifuged at 18,000 rpm for 20 min. The supernatant was then passed over a Dowex or Sephadex column to remove endogenous catecholamines. Analysis of TH versus time revealed an initial 10-15 min period where there was a progressive nonlinear increase in activity after which the reaction rate was linear for up to at least 40 min. This unusual characteristic was independent of protein concentration and the volume of homogenization. To overcome this period of nonlinearity identical sets of samples were incubated for 15 and 30 min and enzyme activity was the difference in activity between these two time points. TH activity was analyzed using a range of BH_4 from 4 to 1000 µM. A double-reciprocal plot of the data revealed three apparent K_m's of 1400, 240 and 7 µM with appropriate V_{max}'s of 7.9, 2.4 and 0.4 pmoles/15 min/µgs respectively. Treatment of striatal supernatant with phosphorylating conditions revealed a different profile on the double reciprocal plot. The high K_m value (1400 µM) was still evident with a similar V_{max}. However we were unable to estimate the contribution of the apparent middle K_m form while the low K_m (7 µM) form of the enzyme now showed a significantly higher V_{max} of 1.0 pmoles/15 min/µg. The present results suggest that phosphorylation converts a form of the enzyme with a K_m of 240 µM to a form with a K_m of 7 µM. The relationship of the forms described under these experimental conditions to those reported previously will be discussed. These results may have important implications not only for the methodology of TH analysis but also for the in vivo mechanism of TH regulation.

234.2 MULTIPLE-SITE PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN SITU: DIFFERENTIAL REGULATION BY ACETYLCHOLINE AND 8-BROMO-cAMP. J.W. Haycock, R.J. George, W.F. Bennett* and J.C. Waymire. Dept. Neurobiol. & Anat., Univ. Texas Med. School, Houston, TX 77025.

Activation of adrenergic tissues causes the release of catecholamines and a compensatory increase in catecholamine biosynthesis. In most cases, activation of tyrosine hydroxylase (TH; E.C. 1.14.16.2), the rate-limiting enzyme in catecholamine biosynthesis, is thought to effect such activity-dependent increases in catecholamine biosynthesis.

Phosphorylation of TH by cAMP-dependent protein kinase (kinase A) increases TH activity, and both the phosphorylation and activation of TH increase up to one mol phosphate/mol TH subunit in vitro. In addition, activation of TH in vivo produces kinetic changes in TH activity similar to those produced by cAMP in vitro. Such data have led to a working model wherein stimulation of catecholaminergic tissues activates kinase A which, in turn, phosphorylates and activates TH. Because TH is rate-limiting, its activation accelerates catecholamine biosynthesis.

Previous results from this laboratory have supported this model: Exogenous cAMP (or 8-bromo-cAMP) increases catecholamine biosynthesis, activates kinase A, and phosphorylates and activates TH in purified, bovine adrenal chromaffin cells. Acetylcholine (ACh--the natural secretagogue of chromaffin cells) also accelerates catecholamine biosynthesis, activates kinase A, and phosphorylates and activates TH. However, several laboratories reported that secretagogues and cAMP analogues activate TH by independent mechanisms; and two cAMP-independent protein kinase systems capable of phosphorylating and activating TH were reported. Thus, to evaluate the possible involvement of multiple kinase systems in the phosphorylation of TH in situ, purified bovine adrenal chromaffin cells were incubated in $^{32}P_i$. ^{32}P incorporation into TH was measured after immunoprecipitation and SDS-PAGE. In some cases, the phosphorylated TH was subjected to tryptic mapping.

On the basis of ^{32}P incorporation, TH was one of the major phosphoproteins. Both ACh and 8-bromo-cAMP increased ^{32}P incorporation into TH. However, as revealed by the tryptic fingerprints, TH was phosphorylated on two distinct phosphopeptides. Interestingly, 8-bromo-cAMP increased ^{32}P incorporation into only one of the sites whereas ACh increased ^{32}P incorporation into both sites. Thus, cAMP-dependent phosphorylation does not appear to be solely responsible for TH phosphorylation in situ. In fact, calcium-, lipid-dependent protein kinase may well be most important in regulating catecholamine biosynthesis in vivo (see next abstract).

234.3 ACETYLCHOLINE-INDUCED PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN BOVINE ADRENAL CHROMAFFIN CELLS MAY BE MEDIATED BY A CALCIUM-, LIPID-DEPENDENT PROTEIN KINASE. R.J. George, J.W. Haycock and J.C. Waymire. Dept. Neurobiology & Anatomy, Univ. Texas Medical School, Houston, TX 77025

Stimulation-dependent phosphorylation and activation of tyrosine hydroxylase (TH) is thought to regulate catecholamine biosynthesis in vivo. And, cAMP-dependent protein kinase activity has been suggested to be responsible for regulating TH. In support of this mechanism, cAMP-dependent protein kinase activates and phosphorylates TH in vitro (up to 1 mol phosphate/mol TH subunit). However, as described in the preceding abstract, TH is phosphorylated at two sites in bovine adrenal chromaffin cells. And, whereas exogenous cAMP increases the phosphorylation of one of these sites, acetylcholine (the natural secretagogue) increases the phosphorylation at both sites. The failure of cAMP to stimulate the phosphorylation of both sites points out that cAMP-dependent protein kinase cannot by itself account for the stimulus-induced phosphorylation of TH.

To identify the mechanism by which ACh induces the phosphorylation of TH we examined the phosphorylation of TH in vitro by endogenous protein kinases in a 100,000 x g supernatant of the chromaffin cell. Both cAMP and calcium stimulated the incorporation of ^{32}P into TH. The addition of phosphatidylserine potentiated the calcium-dependent phosphorylation. The heat-stable inhibitor of cAMP-dependent protein kinase prevented the cAMP dependent phosphorylation but did not inhibit the calcium-, lipid-dependent phosphorylation. The phosphorylation of TH by calcium lipid stimulation incorporated phosphate into two sites on TH as analysed by ^{32}P autoradiography of fingerprints produced by thin layer electrophoresis/chromatography of tyrptic fragments of the enzyme. The calcium, lipid stimulated phosphorylation of TH was distinct from the cAMP-dependent phosphorylation which incorporates phosphate into a single peptide fragment of TH. The sites phosphorylated by calcium-lipid stimulation in vitro appear to be identical to the sites phosphorylated by ACh treatment in situ

The results of these experiments support a role for a calcium-, lipid-dependent protein kinase as the mediator of the in situ phosphorylation of TH in the adrenal chromaffin cells and suggests that the phosphorylation of TH at multiple sites may be important in the regulation of TH in situ.

234.4 INCREASED TRYPTOPHAN HYDROXYLASE ACTIVITY AS AN ADAPTATION TO CHRONIC HYPOXIA. S. H. Feinsilver*, D. Raybin* and E. D. Robin*. (SPON: W. J. McEntee). Department of Medicine, Stanford University and VA Medical Center, Palo Alto, CA 94303

Tryptophan hydroxylase (TPH), an oxygen requiring enzyme, is widely held to be rate limiting for biosynthesis of the neurotransmitter serotonin. The Km of this enzyme for oxygen has been estimated at about 7 torr. Brain PO2 is normally less than 5 torr in some regions; thus mild hypoxia may have dramatic effects on serotonin synthesis.

Using liquid chromatography with electrochemical detection all intermediates of serotonin synthesis were found to be present in murine neuroblastoma cells. Cells were grown to confluence in tissue culture and then exposed to normoxic or hypoxic (PO2 18 to 20 torr) for up to 48 hours. Cell sonicates were assayed for TPH activity using a fluorometric assay for 5-hydroxytryptophan (5-HTP). Activity was significantly increased chronically, but not acutely, by hypoxia:

	TPH Activity (pmol 5-HTP/mg protein/min) + S.E.			
exposure	3 hours	12 hours	24 hours	48 hours
AIR	7.60 ± 0.90	8.01 ± 1.17	6.69 ± 0.36	6.10 ± 0.82
HYPOXIA	8.40 ± 1.29	11.46 ± 0.82	11.45 ± 0.82	9.70 ± 0.37
	n= 10, p NS	n=5, p <.05	n=10, p<.001	n=10, p<.05

Neuroblastoma cells appear to be useful for studying serotonin synthesis in cell culture. Increased TPH activity may represent a compensation for decreased oxygen bioavailability for neuronal serotonin synthesis under hypoxic conditions. A similar effect of hypoxia on tyrosine hydroxylase activity, already reported by this laboratory, suggests a general pattern of neuronal adaptation. These changes may help explain the clinical finding that cerebral functioning is less disturbed by chronic than acute hypoxia.

234.5 EFFECT OF AMFONELIC ACID ON METHAMPHETAMINE-INDUCED CHANGES IN TYROSINE HYDROXYLASE AND TRYPTOPHAN HYDROXYLASE ACTIVITY. C.J. Schmidt* and J.W. Gibb. Department of Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112.

Many of the effects of the non-amphetamine central stimulant, amfonelic acid (AFA), are believed to be due to its action as a potent dopamine (DA) uptake blocker. AFA has been shown to block the behavior induced by amphetamine-like central stimulants as well as the alterations in DA metabolism produced by such agents (Ross, Life Sci., 24:159, 1978; Steranka, Abs., Soc. for Neurosci., 7:207, 1981). We show here that AFA can also block methamphetamine (METH)-induced depression of tyrosine hydroxylase (TH) activity but has no effect on METH-induced decreases in tryptophan hydroxylase (TpH) activity.

Male Sprague-Dawley rats (180-230 gms) were given 10 mg/kg METH (s.c.) every 6 hours for 4 doses. AFA was given simultaneously at doses of 0.1, 0.25, and 1.0 mg/kg (s.c.). Animals were sacrificed 24 hours after the last dose and the striata and cortex were taken. Striatal TH activity was assayed by the method of Nagatsu et al. (1964) and striatal and cortical TpH activity was assayed by a modified ($^{14}CO_2$)-trapping method as described previously by Hotchkiss et al. (1979).

METH reduced striatal TH and TpH to approximately 60% and 50% of controls, respectively. All three doses of AFA completely blocked the decrease in TH activity without altering the change in TpH activity. AFA alone had no effect on striatal TH activity but did cause a significant decrease in TpH activity at a dose of 1.0 mg/kg (to approximately 50% of controls).

Cortical TpH activity was reduced to approximately 30% of control by METH; AFA had no effect on the decrease. AFA alone (0.25 and 1.0 mg/kg) reduced cortical TpH activity to 75% of controls.

The data support the contention that the neurotoxicity due to agents such as METH requires active uptake into monominergic nerve terminals. The inability of AFA to block the effects of METH on the serotonergic system also supports the claim that this agent selectively interferes with DA reuptake. Changes in TpH activity produced by AFA may be due to a direct effect on serotonergic nerve terminals or may alternately suggest an indirect effect via AFA's action on the dopaminergic system. (Supported by USPHS Grants DA 00869 and GM 07579.)

234.6 EFFECT OF DECARBOXYLASE INHIBITION ON MOUSE STRIATAL TYRAMINE. A.V. Juorio* (SPON: D.D. Johnson). Psychiatric Res. Div., Univ., Hospital, Saskatoon, Saskatchewan S7N 0X0, Canada

The discovery that the brain contains small quantities of the p- and m-isomers of tyramine and that these amines cross the blood brain barrier with difficulty suggests that they may be formed within the brain by decarboxylation of p- or m-tyrosine; both reactions are known to occur, though the decarboxylation of p-tyrosine proceeds at a slower rate. This is an investigation of the effects of the subcutaneous administration of some peripheral (carbidopa or benserazide) or central (NSD 1034) inhibitors of L-aromatic amino acid decarboxylase (EC 4.1.1.28). Both carbidopa (5-50 mg/kg) or benserazide (50 mg/kg) produced marked increases in the mouse striatal concentration of p- or m-tyramine; the effect lasted for at least 8 hours. The smaller doses of NSD 1034 (2 mg/kg) produced a moderate reduction (to about 50% of controls) in the concentration of mouse striatal p-tyramine that was observed at 2 hours; higher doses (20 or 400 mg/kg) produced more pronounced reductions (6-14% of controls). The effect of NSD 1034 on m-tyramine followed a different pattern; lower doses (2-20 mg/kg) produced a moderate but significant increase in its concentration (124-131% of controls) while higher doses (400 mg/kg) produced a marked reduction (to 24% of controls). The present findings suggest that the increase in the concentration of peripheral amino acids that occurs after inhibition of peripheral L-aromatic amino acid decarboxylase also include the precursors of the p- and m-isomers of tyramine. It is not possible, however, to ascertain whether phenylalanine and p- or m-tyrosine are increased. The experiments also suggest that there is more than one brain decarboxylase, unless a single enzyme entity possesses different affinities towards p- or m-tyrosine. Studies currently ongoing both in vitro and in vivo are aimed at answering some of these questions. Supported by Sask. Health and the M.R.C. of Canada.

234.7 ASSAY OF PLATELET MONOAMINE OXIDASE USING WHOLE BLOOD, K. Tachiki*, N. Kurtz*, K. Lewis* and A. Kling. (SPON: J. Cummins). Psych. Serv. VAMC Sepulveda Ca 91343 and Dept. of Psych. UCLA Med. Sch. Los Angeles, Ca 90024.

Alterations in activity of platelet monoamine oxidase (MAO) have been reported in the blood of schizophrenic, depressive, or alcoholic patients. The usual assay of platelet MAO requires first an isolation of platelets which is time consuming, affected by the structural lability of platelets, and requires large volumes of blood (approximately 20 ml).

A report (Marshall, E.F. and Campbell, I.C., Biochem. Pharmaco. 26:353, 1977) describing a method for the determination of platelet and plasma MAO in whole blood offered a possible quick assay for platelet MAO. We report a modification of this method which requires less than 0.5 ml of whole blood. Briefly, sonicated whole blood is incubated with ^{14}C benzylamine, sodium borate, pH 9.1, and pargyline (when added) at 37°C in air for 30 min. The reaction was terminated by the addition of 3 N HCL. After setting for 15 minutes, an aliquot of the acidified mixture was transferred to a Clin Elut column (Analytichem International, Inc) and the reaction product ^{14}C benzaldehyde eluted directly into a counting vial with 4 ml of toluene. The reaction rate was linear with time (up to 40 min) and with amount of blood (5 to 100 µl). Under conditions of the assay, maximal inhibition of platelet MAO was achieved with 4 nmoles of pargyline in the assay mixture. No further inhibition in activity was observed when pargyline levels were increased to 32 nmoles. Variability in the assay was 5% (3313 ± 175 dpm, n = 8, mean ± s.d.). Assay blanks were 162 dpm which under the standard assay conditions would represent an activity of 1.3 nmoles product formed per hr per ml whole blood. Thus, the lower sensitivity limit (i.e., twice blank) of activity is 2.6 nmoles product per hr per ml blood.

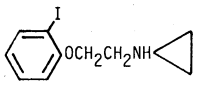
Using this method, chronic paranoid schizophrenics had significantly lower platelet MAO activity than age matched, non-paranoid schizophrenics (75 ± 10, n = 10 vs. 192 ± 18, n = 9 nmole product/gm prot./hr; means ± s.e.m; $p < 0.001$). These findings are in agreement with those reported previously (Potkin, S.G. et al., N. Eng. J. Med. 298:41, 1978). Preliminary findings from patients treated with MAO inhibitors showed plasma MAO activity to be strongly inhibited by phenelzine whereas plasma activity was not appreciably affected by treatment with tranylcypromine. These findings correlate with reported results (Robinson, D.S., Biochem. Pharmacol. 17:109, 1968). The similarity in results with literature reports indicate this method can be a clinically important assay for platelet MAO.

234.8 PURIFICATION OF ENDOGENOUS INHIBITORS OF MAO FROM PLASMA AND CNS TISSUES. C.T. GIAMBALVO AND R.E. BECKER. RI Psychiatric Research and Training Center, Brown University and the University of Rhode Island, Cranston, RI 02920.

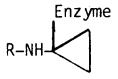
We have previously demonstrated the presence of an endogenous inhibitor of MAO in crude plasma of humans. We report here that the modulator is also present in CNS tissues (CSF and rat brain). The modulators were purified (250-8500 fold) through gel chromatography on Sephadex G-50 and ion-exchange chromatography on Dowex 50 W - X 8 (H^+). Results showed that there are 2 modulators of MAO-A, with molecular weights of >35,000 and about 3500 daltons. These tissues contain 3 modulators of MAO-B with molecular weights of >35,000, 7000 and 14,000 daltons respectively. All these modulators inhibited in a non-competitive manner, by increasing the Km. The MAO-A inhibitors were 3 to 6 times more potent than the MAO-B inhibitors. These modulators are thermostable acidic proteins that are susceptible to trypsin treatment but not to heating at 95° C for 5 minutes.

234.9 SELECTIVE, IRREVERSIBLE INHIBITION OF TYPE A MONOAMINE OXIDASE BY N-[2-(o-IODOPHENOXY)ETHYL]CYCLOPROPYLAMINE HYDROCHLORIDE (LY121768). Ray W. Fuller, Susan K. Hemrick-Luecke* and Bryan B. Molloy*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

LY121768 was a potent inhibitor of the oxidation of serotonin, a substrate for type A monoamine oxidase (MAO), by MAO from rat brain mitochondria in vitro. The concentration of LY121768 that inhibited serotonin oxidation by 50% was 4 x 10^{-10}M in the standard assay, whereas 2800 times higher concentrations were required for inhibition of the oxidation of phenylethylamine, a substrate for type B MAO. This selectivity is 3 times greater than for the corresponding compound (LY51641) with a chlorine in place of the iodine substituent. The in vitro inactivation of type A MAO by LY121768 was time-dependent, was not reversible by dialysis, and was prevented by inhibiting MAO reversibly with harmaline, indicating that enzyme action was necessary for the inactivation. In rats, LY121768 inhibited type A MAO in brain in vivo with an ED_{50} of 0.39 mg/kg i.p. when MAO activity was measured 1 hr after drug injection. At 1, 3, 5 and 7 days after the injection of a single 10 mg/kg i.p. dose of LY121768, type A MAO in brain was inhibited by 78%, 52%, 56% and 34%, respectively, whereas type B MAO was not inhibited at any of these times. Associated with this inhibition of type A MAO were significant increases at all times in hypothalamic concentrations of norepinephrine, epinephrine and dopamine, and decreases in the concentrations of the dopamine metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, measured in cerebral hemispheres. N-Cyclopropyl-arylalkylamines of this type are thought to be "suicide substrates" that are acted upon by MAO to form reactive cyclopropanone imines, which then covalently attach to a functional group on the enzyme (Silverman and Hoffman, J. Am. Chem. Soc. 102, 884, 1980), forming a structure as shown in the inset. Synthesis of LY121768 containing an appropriate radionuclide of iodine could provide a useful means of labeling type A MAO selectively for scintigraphic imaging or other research purposes.



LY121768



234.10 INHIBITION OF BOVINE BRAIN CHOLINE ACETYLTRANSFERASE BY MEMBRANE PHOSPHOLIPIDS. C. A. Kasal, C. W. Kirsting*, S. M. Hurd*, W. O. McClure, and R. Ryan*. Neurobiology, Univ. of Southern California, Los Angeles, CA 90007.

The interaction of choline acetyltransferase (CAT) with lipid components of cellular membranes was examined in vitro using liposome preparations of selected phospholipids, and CAT partially purified from bovine caudate nuclei by the method of Ryan and McClure (Biochem. 24, 5357, 1979). Several lipids were found to inhibit enzyme activity while others had no apparent effect. Liposome preparations (.08 mg lipid/ml 10mM citrate phosphate buffer, pH 7.3) of either cardiolipin (CL), CDP-digliceride (CDP), phosphatidic acid (PA), phosphatidyl glycerol (PG), phosphatidyl inositol (PI) and phosphatidyl serine (PS) inhibited CAT activity in varying degrees (CL 100%; CDP 70%; PA 68%; PG 50%; PS 34%) when compared to control values. Liposomes composed of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl ehthanolamine plasmalogen and their lyso-derivatives, as well as sphingomylin and 1,2 diglyceride exhibited no inhibitory effect. In all cases, the observed inhibition was blocked by the presence of the substrate acetyl-CoA, however, the second substrate, choline, did not protect the enzyme from inhibition suggesting that all inhibitory lipids are acting at or near the acetyl-CoA binding site. Furthermore, comparison of the net charges present at the polar head of each inhibitory lipid at neutral pH, suggest that a net negative charge may promote inhibitory interaction.

The observed in vitro inhibition suggests a possible physiological role for membrane lipids in the regulation of CAT. Further aspects of this lipid-enzyme interaction will be presented.

Work supported by NIH grant AG-01896 and Nelson Research and Development, Irvine CA.

234.11 A ROLE FOR CALCIUM IN THE REGULATION OF GLUTAMATE DECARBOXYLASE. Barry I. Gold. Dept. Pharmacol. Uniformed Services Univ. Sch. Med. Bethesda, MD 20814

We recently hypothesized that the binding of Ca^{2+} to the outside of neuronal membranes was involved in the regulation of glutamate decarboxylase (GAD) (Gold & Huger, Biochem. Pharmacol. 31: 882, 1982). I now report results of experiments in which I tested that hypothesis by measuring GABA synthesis (SYN) in brain slices incubated in media designed to alter Ca^{2+} fluxes or Ca^{2+} sequestration.

Slices of corpus striatum, dissected from male rats were prepared as described in our previous reports (Gold & Huger, ibid.). In preliminary studies, slices were suspended in 5 ml Krebs-Ringer-bicarbonate medium (KRB). After a 10 min incubation period, [2,3-^{3}H]glu (0.5mM final) was added and slices were incubated another 10 min. The reaction was halted by acidification and the slices were homogenized with a Polytron. Following a low-speed centrifugation, [^{3}H]GABA was isolated from the supernatants by cation-exchange chromatography and radioactivity was estimated by liquid scintillation spectrometry. Later studies included a preincubation and a wash. Slices were placed into polypropylene baskets with porous bottoms lined with discs of Kimwipes. The baskets were in glass vials containing 5 ml normal KRB or KRB which contained 50mM K^+, both in the presence or absence of a drug. After 10 min the baskets were transferred to fresh KRB for a 10 min repolarization. They were then transferred again to fresh medium which contained 0.5 mM [2,3-^{3}H]glu. Subsequent steps were as in the preliminary studies.

In preliminary studies, the electron transport inhibitor antimycin A inhibited [^{3}H]GABA SYN 39% at 20μM. Similarly, the uncoupler of oxidative phosphorylation, 2,4-dinitrophenol inhibited [^{3}H]GABA SYN 68% at 0.4mM. Both drugs increase intracellular concns of ionized Ca^{2+} by promoting leakage of mitochondrial Ca^{2+} (Alnaes & Rahamimoff, J. Physiol. 248:285,1975). Neither Ruthenium red (10μM) nor lidocaine (1.0mM) affected [^{3}H]GABA SYN under these conditions.

Preincubation in high K^+ increased [^{3}H]GABA SYN 40-90% and this stimulation was not blocked by 1.0mM lidocaine. [^{3}H]GABA SYN was stimulated when slices were preincubated in lidocaine alone. Preincubation in 0.5mM 8-(diethylamino)octyl 3,4,5-trimethoxybenzoate-HCl (TMB-8) alone had no effect, however, it partly reversed the stimulation by high K^+. Preincubation in verapamil (100μM) alone was without effect and it did not reverse the stimulation by K^+.

These results are consistent with our hypothesis that Ca^{2+} is involved in the regulation of GAD activity, however, they suggest that both intra- and extracellular Ca^{2+} are important.

234.12 INHIBITORS AND INACTIVATION-PROMOTERS OF GLUTAMATE DECARBOXYLASE T.G. Porter*, S.B. Martin*, and D.L. Martin. Center for Labs and Research, New York State Health Dept., Albany, NY 12201

Previous studies (Meeley et al.,Brain Res.Bull. 5, Suppl. 2,57, 1980) have shown that brain glutamate decarboxylase (GAD) undergoes substrate-promoted inactivation which has been postulated as a regulatory mechanism for controlling GABA synthesis in vivo. The present studies were performed to distinguish between certain compounds which enhance this substrate-promoted inactivation (inactivation-promoters) and those which compete directly with the substrate at the active site. Inactivation-promoters are characterized by their slow action, their requirement for high concentrations of glutamate and appear likely to act at a site distinct from the active site of the enzyme. Competitive inhibitors act immediately, and their effects are overcome by high concentrations of glutamate. Since glutamate-promoted inactivation appears to occur by a branch in the normal decarboxylation mechanism, competitive inhibitors should also reduce inactivation by glutamate by preventing it from entering the active site.

ATP and a number of seemingly structurally unrelated compounds (e.g. 4-deoxypyridoxine phosphate, 5-phosphorylribose 1-pyrophosphate, inositolhexasulfate) promote inactivation by glutamate. The concentration of glutamate required to inactivate GAD by 50% in 20 min was reduced from about 1.5 mM to 0.5 mM by addition of 50 μM ATP. Despite its inactivation promoting effects, 50 μM ATP had no significant effect on the V_{max} or K_m for glutamate when tested in a short (5 min) assay showing that it does not block access of glutamate to the active site.

Twenty conformationally-restricted analogues of glutamate including hydroxybenzoic acids, benzene tricarboxylic acids, benzene tetracarboxylic acids, pyridine dicarboxylic acids and pyran dicarboxylic acids were tested for their ability to inhibit rat-brain GAD. Chelidonic acid, chelidamic acid, 2,6 pyridinedicarboxylic acid, gallic acid and 3,4 dihydroxybenzoic acid were found to be the most potent inhibitors. Kinetic analysis of chelidonic and chelidamic acid confirmed that these were competitive inhibitors with K_i values of 1.2 μM and 33 μM respectively. Thus chelidonic acid is one of the most potent competitive inhibitors of GAD known. At a concentration of 7.5 μM, chelidonic acid retarded the rate of inactivation of rat brain GAD by 5 mM glutamate, an effect opposite that seen with inactivation promoters.

These results demonstrate a clear mechanistic difference between the effects on GAD of substrate analogs such as chelidonic acid and inactivation-promoters such as ATP.

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235.1 LASER ABLATION OF AVIAN NEURAL CREST CELLS. James Coulombe* and Marianne Bronner-Fraser. (SPON: M. Cahalan) Department of Developmental and Cell Biology and Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

One of the earliest methods for studying the derivatives arising from the neural crest in avian embryos was by surgical ablation of the crest (Yntema and Hammond, J. Exp. Zool. 100: 237, 1945). Unfortunately, such surgical procedures often cause deformities which confound interpretation of the results. In order to avoid these problems, we have used laser technology to ablate the neural crest. Laser irradiation makes it possible to reproducibly remove crest cells without disrupting the integrity of the embryo.

Prior to irradiation, a window is made over the chicken embryo (Stage 12-14). The embryos are stained with the vital dye neutral red and viewed by reflected light. For the irradiations, we use the Q switched, frequency doubled output (352 nm) of a YAG:Nd laser, focused onto the dorsal neural tube. During the irradiation, the specimen is moved through the microbeam by means of a motorized stage whose movement is coupled to the firing of the laser. A laser pulse is delivered to the neural tube every half micron of stage travel. Typically, six or more somite lengths of neural tube are irradiated. After ablation, the embryos are returned to the incubator, fixed at the desired time, and prepared for histology.

A few seconds after irradiation, perturbation of the dorsal neural tube is evident. Embryos fixed at this time showed cell death only on the dorsal aspect of the neural tube. Most embryos allowed to survive for one day following irradiation showed little visible deformity other than necrosis at the dorsal surface of the neural tube. As an assay of the effectiveness of the laser ablation, we observed the presence or absence of dorsal root ganglia (DRG). The dorsal root and sympathetic ganglia are first observed in normal embryos at five days of incubation (corresponding to two days post-irradiation). In our preliminary results, nine animals were fixed two days following ablation. Of these animals, three showed complete absence of DRG's at the level of the ablation; another embryo had unilateral absence of DRG's with reduction in ganglion size on the contralateral side. Four other embryos showed marked reduction in size of the ganglia and only one showed no visible effect. In general, the embryos had no apparent malformations except for slight neural tube abnormalities.

These results indicate that laser irradiation of the neural crest will be useful as a reproducible method for removing the neural crest without serious deformation of the embryo.

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235.2 THE EFFECTS OF SURFACE CHARGE ON DISTRIBUTION OF LATEX BEADS ALONG THE VENTRAL NEURAL CREST PATHWAY. Marianne Bronner-Fraser. Dept. of Physiology & Biophysics, University of California, Irvine, CA 92717.

Neural crest cells migrate extensively in the embryo along precise pathways. In a previous study, it was observed that latex beads distribute similarly to neural crest cells along the ventral neural crest pathway after injection into the adjacent somites. When the bead surface is coated with fibronectin, however, the coated beads remain at the site of implantation and do not distribute along the neural crest route (Bronner-Fraser, Devel. Biol. 91: 50, 1982). In the present study, latex beads of varied surface charge were introduced into embryos at the time of crest migration in order to determine the effects of charge on bead localization.

For these studies, two types of cationic beads were prepared. First, polystyrene beads (Polysciences, Inc.) were coated with poly-D-lysine by incubating plain beads with poly-D-lysine (1 mg/ml H_2O). A second type of cationic bead was produced by covalently reacting hydrazine dihydrochloride with carboxylated beads (Dow Diagnostics). Latex polystyrene beads have an inherent negative surface charge. Beads with neutral surface charge were produced by coating the bead surface with poly-tyrosine (1 mg/ml in phosphate saline).

As previously reported, latex beads with negative surface charge distributed along the ventral neural crest pathway and localized adjacent to endogenous neural crest sites. The neutral poly-tyrosine coated beads also translocated ventrally and localized in the vicinity of the sympathetic ganglia, ventral nerve cord, or dorsal aorta; these sites are normally populated by neural crest cells. These results are in contrast to those found for positively charged beads. The poly-D-lysine coated beads did not translocate along the ventral neural crest route but remained at the site of implantation; most frequently, the beads localized in the dermamyotomal remnant of the somite. Similarly, covalent positively charged beads did not move along the pathway but remained at the injection site.

These results show that both negatively charged and neutral latex beads translocate ventrally; positively charged beads, on the other hand, mimic the behavior of fibronectin coated beads, and do not translocate along the ventral pathway after injection into the embryonic somites. Unlike the cationic beads, fibronectin is negatively charged at physiological pH. These findings indicate that a broad range of adhesion mechanisms (including electrostatic interactions) can serve to restrict access to neural crest sites of localization.

Supported by USPHS Grant HD-15527-01 and by Basil O'Connor Starter Research Grant 5-312 from the March of Dimes.

235.3 DEVELOPMENT OF THE RELATIONSHIP BETWEEN DORSAL ROOT AFFERENTS AND THE LATERAL MOTOR COLUMN OF THE BULLFROG SPINAL CORD. F.J. Liuzzi, M.S. Beattie and J.C. Bresnahan. Dept. of Anat. and Div. of Neurosurg., and Neuroscience Research Lab., Ohio State Univ., Columbus, OH 43210.

The development of primary afferent input into the lateral motor column (LMC) in the lumbar enlargement of Rana catesbeiana was studied as a function of hindlimb development. Identification of pre- and post-synaptic elements was achieved by the simultaneous injury-filling of the ninth dorsal and ventral roots with horseradish peroxidase (HRP) in adult and larval bullfrogs. In the adult, primary afferents were seen to have two fields of termination. One, in the superficial dorsal horn, an area possibly homologous to the mammalian substantia gelatinosa receives fine caliber primary afferent input. The second in the deep dorsal horn and intermediate gray receives large caliber primary afferent input. This second area overlaps the dorsal arborization of LMC motoneuron dendrites. Additionally, some large caliber primary afferent fibers enter the lateral motor column. Light and electron microscopy show that numerous en passant terminals on motoneuron dendrites are formed by primary afferent fibers in the more ventral terminal field while the large caliber primary afferents that enter the LMC synapse upon motoneuron primary dendrites and somata. Both the axodendritic and the axosomatic synapses are symmetrical with small (30-35nm), agranular, spherical vesicles. In the tadpole, the development of the primary afferent distribution occurs concurrently with the development of the dorsal dendritic arborization of the LMC motoneurons. The adult pattern of primary distribution is attained by hindlimb stage XVII (Taylor and Kollros, 1946). By this stage numerous en passant synapses are observed between HRP-labelled primary afferent axons and LMC motoneuron dendrites. At this stage, however, the number of axosomatic synapses formed by primary afferent axons on LMC motoneurons is still considerably fewer than in the adult frog. This may reflect the transition from a strictly aquatic animal at stage XVII to a true amphibian which spends at least part of its life on land. (Supported by NIH grants NS-14457 and NS-10165, and a grant and Presidential Fellowship from the Graduate School, The Ohio State University.)

235.4 MIGRATING MOTONEURONS AND THEIR RELATIONSHIP TO DEVELOPING DORSAL ROOT AFFERENTS IN THE BULLFROG TADPOLE SPINAL CORD. M.S. Beattie, F.J. Liuzzi and J.C. Bresnahan. Div. of Neurosurg. and Dept. of Anat. and Neurosci. Res. Lab., Ohio State Univ., Columbus, OH 43210.

Previous studies have shown that labelling of ventral roots with horseradish peroxidase fills neurons located between the lateral motor column and the ventricular zone in developing anuran tadpoles (Farel and Bemelmans, 1980). These neurons have been interpreted as motoneurons in the process of migration toward the LMC. In the present study HRP was applied to the cut ninth dorsal and ventral roots in Rana catesbeiana tadpoles from stages X to adult (staging of Taylor and Kollros, 1946) to provide for simultaneous labelling of motoneurons and dorsal root primary afferent axons. Transverse and parasagittal sections were cut on a vibratome and processed for sequential light and electron microscopic analysis.

Presumptive migrating motoneurons were labelled in stage X to stage XIV tadpoles. In stages X-XIII these neurons were located in a pattern extending ventrolaterally from the ventricular zone along the border of the ventral horn and then dorsolaterally into the lateral motor column (LMC). In three stage XIV cases, a prominent pattern of labelled neurons was located more dorsally, through the intermediate gray, extending in an arc from the ventricular zone to the dorsal aspect of the LMC. In these cases, labelled dorsal root primary afferent axons extended into the zone occupied by the dorsal band of migrating motoneurons, and many axonal swellings were observed in close apposition to the somata and fine medial-lateral processes extending from them. Primary afferent axons were not observed in the region of the ventral band of migrating cell bodies.

Electron microscopy revealed HRP-labelled somata with thin cytoplasm surrounding a large nucleus. Synapses were occasionally observed on these neurons, including terminals with small, round agranular vesicles. In addition, labelled dorsal root afferent axons exhibited synaptic specializations which contacted other labelled processes in the neuropil, some of which appeared to represent the thin processes of the labelled motoneurons. Further examination with electron microscopy will be required to determine the extent to which dorsal root afferents make contact with migrating motoneurons. The coincidence of primary afferent penetration into the intermediate spinal gray and the presence of a dorsal migratory pathway of motoneurons suggests that these two systems may have functional interactions prior to the establishment of their mature relationship after the completion of the development of the LMC. (Supported by Grants NS-14457, NS-10165, and a grant and Presidential Fellowship from the Graduate School, The Ohio State University.)

235.5 LARGE CIRCUMFERENTIAL CELLS OF THE DEVELOPING RANA CATESBEIANA SPINAL CORD ARE LABELLED AFTER HRP APPLICATION TO LATERAL FUNICULUS. H.L. Campbell, F.J. Liuzzi, M.S. Beattie and J.C. Bresnahan. Dept. of Anat. and Div. of Neurosurg. and Neurosci. Res. Lab., Ohio State Univ., Columbus, OH 43210.

Neural elements contributing to the lateral and ventral funiculi at different stages during hindlimb development in bullfrog tadpoles were examined by placing chips of dessicated horseradish peroxidase (HRP) into restricted lesions of the white matter rostral to, or in the lumbar enlargement. These experiments were performed in a series of tadpoles at stages X to XIX of Taylor and Kollros (1946). Five adult animals were also used.

HRP placements into the lateral and ventral funiculi results in diffuse filling of axons which enter the spinal gray, as well as some spinal gray neurons. This report will focus on a unique cell type which has been observed only in tadpoles in our material (stages X-XIX), and is seen mainly contralateral to HRP placements at levels remote from the lesion. The somata (10-14μm, dia.) are located at the gray-white border and have two large, smooth primary processes (4-5μm, dia.) which sweep around the circumference of the gray matter. The diameter of these processes is remarkably constant along their course which circumvents the spinal gray dorsally and ventrally. Many circumferential cells extend a large process medially across the superficial dorsal horn to the midline, and a second process which follows the border of the ventral horn to the ventral midline (see illustration). A very few smaller processes emanate from the medial aspect of the dorsal processes as they approach the midline. Almost no other branches of the large processes are present.

In one micron thick plastic sections, some of these large ventrally extending processes were myelinated. Electron microscopic observations of one of the dorsally extending processes in a stage XIX case showed it to be dendritic with large numbers of synaptic contacts.

These cells may be similar to the arcuate neurons described in the larval salamander by Herrick and Coghill (1915). The precise time at which they disappear late in the development of the frog spinal cord, as well as their function(s) remains to be determined, and electron microscopic analysis is underway to characterize their synaptic inputs. (Supported by Grants NS-14457, NS-10165, and a grant and Presidential Fellowship from the Graduate School, The Ohio State University.)

235.6 GOLGI, IMMUNOCYTOCHEMICAL AND ELECTRON MICROSCOPIC EVIDENCE FOR THE RADIAL ORGANIZATION OF THE DEVELOPING DENTATE GYRUS IN RHESUS MONKEY. M.F. Eckenhoff and P. Rakic, Sec. of Neuroanat. Yale Univ. School of Medicine, New Haven, CT 06510.

The cytological organization of the dentate gyrus (DC) was analyzed in rhesus monkey hippocampus at various embryonic (E) and postnatal (P) ages with the rapid Golgi method, PAP immunocytochemical staining, using a specific antibody to glial fibrillary acidic protein (GFA) and electron microscopy (EM). At all developmental ages, the dentate plate has distinct radial organization that is expressed by the vertical stacks of immature granule cells separated by elongated radial fibers. These fibers extend from cell bodies near the ventricle and in the hilus of the DG and terminate at the pial surface with endfeet. Their glial nature is confirmed by GFA-positive reactivity seen first clearly at E70. At this stage, GFA stained fibers curve and fan out in a radial fashion from the ventricular zone to the pial surface. In cresyl violet counterstained sections they are aligned with columns of unlabeled granule neurons. Furthermore, EM analysis shows that granule cells, as well as many neurons still migrating from the ventricular zone, are closely opposed to processes of radial glial cells. The radial organization of cell bodies in the granular layer is even better exposed by GFA immunoreactivity at E90 and E125. Glial processes exit from their somas situated in the polymorphic layer, penetrate radially between cells in the granular layer and traverse the molecular layer to terminate at the pial surface. At the EM level, each radial unit consists of granule cell bodies in different stages of maturation with the more mature cells located superficially and the less mature cells at progressively deeper levels. Furthermore, columns of electronlucent glial fibers separate the individual columns of granule cell bodies. The number of radial glial fibers in the granular layer diminish at E149, P3, P17 and P34 when the radial organization becomes less distinct due to shifting and rotation of the DG at these ages. At the same time, large numbers of astrocytes become visible in the polymorphic layer and outer molecular layer. Dividing and immature granule cells are situated in deeper strata of the granular layer at these ages observed with EM, confirming local genesis of granule cells in the rhesus monkey at late ages (Rakic and Nowakowski,J.C.N., 196:99,1981). In summary, the developing DG is organized as a series of radial columnar units which basically resemble those in the developing neocortex (Rakic, J.C.N.,145:61,1972). At initial stages, each DG unit consists of cells which have a common precursor in the ventricular zone and this radial organization may depend on the transient presence of elongated glial cells. Supported by NS14841.

235.7 FIBER ABNORMALITIES OF THALAMUS AND MIDBRAIN IN REELER MUTANT MICE. D.O.Frost, V.S.Caviness, Jr., and G.M. Sachs, Yale Medical School, New Haven,CT. and E.K. Shriver Center, Waltham, MA

The reeler mutation in mice produces in all cortical structures of the forebrain and cerebellum correlated abnormalities in the radial positions of neuronal somata and associated efferent and afferent fiber systems. We now report that despite a normal organization of neuronal somata in the reeler thalamus and midbrain, there are anomalies of axon trajectory in those structures.

Silver Staining of normal axons reveals: 1) Axon facicles from the brachium of the superior colliculus (bsc) penetrate the rostral edge of the stratum griseum superficiale (SGS) of the superior colliculus (SC) and pass caudaly at all depths between the surface of SC and the bottom of the stratum opticum (SO). 2) Depending upon the coronal level examined, the thalamic external medullary lamina (eml) is attenuated or absent. The dorsal nucleus of the lateral geniculate body (LGd) is cut into dorsomedial and ventrolateral segments by an anomalous, longitudinally oriented axon sheet that, like eml, is continuous ventrally with fiber tracts forming the ventral thalamic border. Dorsally, this sheet merges with the optic tract (ot). 3) Axon fascicles in the posterior (Po) and medial geniculate (MG) nuclei normaly form orderly curvilinear arrays; in reeler these fascicles are intertwined and less regularly oriented and spaced.

The anomalous fascicles in SGS contain retinofugal axons-contralateral enucleation or partial retinal lesions produce axonal degeneration in these fascicles, and horseradish peroxidase (HRP) injections into bsc or ot label them. Golgi-like HRP filling of single ot fibers shows that after passing caudally in SGS for varying distances, as in normal mice, thinner axons approach their zone of termination just beneath the surface of SC from below. Thicker axons terminate deeper; unlike their normal homologues they may approach their zone of termination from above or below. The radial and tangential spread of individual axonal arbors, the degree of axonal ramification and the density and size of terminal boutons all appear normal.

The abnormal trajectories of reeler retinotectal axons may be due to the stabilization of a superficial population of fibers that disappears during normal development. This does not appear to alter the connections formed by the retinofugal axons.

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235.8 RADIAL ORGANIZATION OF THE EMBRYONIC CEREBRAL WALL IN MICE: DEPLOYMENT OF SOME AFFERENT AXONAL SYSTEMS IN RELATION TO THE INTERMEDIATE ZONE - SUBPLATE INTERFACE. J.E. Crandall and V.S. Caviness, Jr. Dept. Neurology, Mass. Gen. Hospital and E.K. Shriver Center, Boston, MA 02114

The distribution of corticothalamic (CT), thalamocortical (TC), monoaminergic (MA) and callosal (CC) fiber systems are defined with respect to the cortical plate (CP), subplate (SB) and intermediate zone (IZ) in the murine cerebral wall on embryonic day 16 (E16). This developmental age is within 24 hours of the formation of the callosal decussation and 48 hours of the formation of the CP.

The cingulate bundle, already a conspicuous rostro-caudally aligned fascicle at the medial aspect of the hemisphere at E16, defines the plane of the external sagittal stratum (ESS). Though attenuated, this fiber plane is readily discernible in general cell stains as it extends laterally from the cingulum under the neocortex. Because the ESS lies at the interface of the mature cortex and central white matter, it is considered for the purposes of the present analysis to be the SB-IZ boundary. Medially to laterally arching fiber arcades pass across the IZ between the ESS and subcortical structures. More obliquely coursing fibers cross the IZ from lateral to medial from the ESS toward the callosal decussation.

Hodologic analyses based upon retrograde and anterograde transport of HRP, monoamine histofluorescence and Golgi impregnations establish that all fiber systems ascending between cortical and subcortical levels course tangentially for a variable distance in the ESS. The TC and CT fibers, which share the same trajectories, are the dominant axonal system in this stratum and serve to confirm its identity as the fetal counterpart of the ESS. Fibers of MA systems are more sparsely concentrated in this fiber plane. TC, CT and MA axons are included in the fiber arcades which cross the IZ. TC and CT axons enter the internal capsule together. Those MA axons directed to the convexity of the hemisphere ascend somewhat more laterally and inferiorly from the external capsule. Terminals of the TC and MA axons as well as those of callosally derived axons ramify in overlapping fashion within the SB. Axons of all three systems also penetrate the lower fringes of the cortical plate and may influence trophically neuronal growth and differentiation proceeding rapidly at this interface.

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235.9 ARREST OF NEURONAL MIGRATION IN REELER NEOCORTEX: A GOLGI-EM ANALYSIS OF NEURON-GLIAL RELATIONSHIPS. M.C. Pinto-Lord*, P. Evrard*, V.S. Caviness, Jr., Dept. Neurology, Mass. Gen. Hospital and E.K. Shriver Center, Boston, MA 02114

Reeler is an autosomal recessive mutation in mice which causes inversion in the positions of neocortical neurons relative to their sequence of migration. Whereas the relationship is normally "inside-out", it is "outside-in" in the mutant. The migration of neurons along radial glial fibers (RGF) in E17 reeler embryos is examined in electron micrographs of tissue in which RGF are impregnated by the Golgi-gold-toning method of (Fairen, A., et al., J. Neurocytol., 6:311,1977). RGF are richly deployed in the mutant cerebrum with a normal interfiber interval of 10-15 um. The following anomalies are characteristic of the fibers in the mutant: sparsely scattered adhesions between transcortical segments of adjacent fibers, diminished richness of fiber branching in the sub-pial zone and gaps in the limiting glial membrane formed by terminal expansions of the fibers (cf., Derer, P., Neurosci. Lett., 13:195,1979). The configuration of the migrating neuron and its relation to the surface of the radial glial fiber is normal as the cell crosses the intermediate zone. Its leading process is normally tapered and the young cell is apposed throughout its length to the surface of the fiber. In the depths of the cortical plate, however, anomalies of cell configuration and in the relation of the young neuron to the surface of the radial glial fiber are correlated with migration arrest. The leading process is observed to become blunted, bifurcated and is, apparently, unable to interpose itself between the surfaces of post-migratory cells and the RGF. Multiple small processes emerge from the soma. These and bifurcations of the leading process become insinuated between apposed surfaces of post-migratory neurons. Although upward movement is halted the soma of the young cell remains extensively apposed to the surface of the fiber, a phenomenon not observed in the normal animal where contact with the RGF is largely surrendered shortly after termination of migration. This set of observations suggests that abnormal surface adhesions between post-migratory neurons and RGF obstruct the ascent of migrating neurons in reeler. The molecular mechanism of adhesion and its relation to mutation at the reeler locus are unknown.

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235.10 CONNECTIONS OF THE NEWBORN RAT OLFACTORY BULB. M.T. Shipley and G.D. Adamek. Dept. Cell Biology & Anatomy, Northwestern Univ. Med. Schl., Chicago, IL 60611.

The rules and mechanisms underlying the formation of brain connections is important to an understanding of normal adult brain function as well as birth and developmental neural defects. Knowledge of this kind has been hard to obtain because of problems associated with accurately placing small amounts of pathway tracers in a specific brain site at different developmental stages. We have surmounted these problems and chosen to study the ontogeny of the connections of the rat main olfactory bulb (MOB) because this structure is directly accessible at all stages of development. The efferents of the MOB and its peripheral and centrifugal afferents can be demonstrated by a single injection of wheat germ agglutinin conjugated to horseradish peroxidase (WGA/HRP). These connections are well characterized in the adult and include centrifugal afferents from a number of transmitter-specific pathways ranging from the ventral forebrain to the pons and medulla. We report here on the connections of the MOB for P-2 rats (birth = P-1) using WGA/HRP tracing, acetylcholinesterase histochemistry (AChE) and fluorescence for monamines.

At P-2 the MOB's efferents are immature but have reached nearly all adult targets except the olfactory tubercle which is only partly innervated. Projections are heavier to posterior parts of the primary olfactory cortex (POC) and the entorhinal area. This may reflect an earlier maturation of mitral vs. tufted cell outputs. Primary afferents from the olfactory epithelium appear to reach all of the MOB but are more dense in the rostral part of the bulb. Centrifugal afferents (CAs) are quite immature and some are non-existent. In the adult the anterior olfactory nucleus, the most proximal structure to the bulb, provides one of the richest sources of MOB-CAs. At P-2 this system is very weak, proportionally fewer cells are labeled than in the more distal POC. The nucleus of the diagonal band (NDB) contains some labeled cells and similar numbers are AChE positive but there are no AChE+ terminals in the MOB. The nucleus of the lateral olfactory tract adjacent to NDB has no labeled cells at P-2. The locus coeruleus (LC) has many labeled cells; fluorescence studies of LC fibers are in progress. Only a small fraction of raphe cells is labeled and no hypothalamic or amygdaloid CAs appear to be present.

The connections of the P-2 rat MOB are very immature. Biochemical synaptic maturity appears to lag behind connectional maturity. Distance from the bulb does not appear to govern the time or sequence of connection formation. Older and younger (P-1 and fetal) animals are currently under investigation. (Supported by NIH NS 16083 to M.T.S. and NSF BNS 8117075 and NIH NS 14663.)

235.11 SPATIAL LOCATION OF ACTIVE NEURONS IN CORTICAL BARRELS OF NORMAL AND WHISKER-DAMAGED MICE. D. Durham and T.A. Woolsey. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

The barrels of the mouse SmI cortex are "isomorphic" to the whiskers on the contralateral face from which they receive sensory input. If the whiskers are damaged in the first week of life the architecture of the barrels is altered in a systematic fashion. In this study we examined the relationship between the cortical cytoarchitecture and neuronal activity directly with the ^{3}H-2DG method (Durham et al., J. Neurosci. 1:519, 1981). Adult animals which either had no whisker damage or whose middle row of whiskers was destroyed by electrocautery on each of the first 5 days of life were studied. After injection of 2-4 mCi ^{3}H-2DG the animals were allowed to explore their cages freely to provide whisker stimulation prior to sacrifice. All subsequent processing was as described previously. In particular the sensory periphery was evaluated histologically and the cortex was cut in a plane parallel to layer IV to visualize the barrels. The barrel fields were reconstructed with a camera lucida and from photomicrographs of the serial sections. From similar dark-field photomicrographs and with the camera lucida labeled cell somata were identified and plotted in relation to the cortical cytoarchitecture. In normal animals the barrels receiving inputs are heavily labeled: the label is present in neurons and neuropil. Adjacent barrels associated with absent whiskers have much less label in the neuropil and the cells which are occasionally labeled tend to be located in the barrel septa or adjacent to the barrels receiving normal inputs. In the whisker-damaged animals similar patterns of neuronal labeling were seen. In particular there is no increase in labeling of neurons located in the altered cortex associated with the damaged whiskers. These results confirm and extend our earlier physiological studies using similarly prepared animals. Namely the cortical barrels correspond precisely to functional boundaries even if the barrel architecture is altered experimentally (Simons et al., Soc. Neurosci. 6:638, 1980). What this study adds is precision to the conclusion since the exact location of active neurons has been determined microscopically in the context of a defined cortical cytoarchitecture.

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235.12 OBSERVATION AND ANALYSIS OF MITOTIC ACTIVITY IN THE DEVELOPING CAT RETINA. D.H. Rapaport and J. Stone. School of Anatomy, University of New South Wales, Kensington, N.S.W. AUSTRALIA.

The retinal whole mount has served as a useful preparation in which to study the topography of retinal ganglion cells at the vitreal surface of the retina. The mitosis of neuroblasts in the developing retina occurs at the ventricular surface, which is closely apposed to the pigment epithelium, and in the adult becomes the layer of receptors. By whole-mounting retinae from fetal animals with the ventricular layer (as opposed to the vitreal layer) up we could observe the distribution of cells in active mitosis at the time the kitten was sacrificed.

Retinae from fetal cats of known gestational age were fixed by perfusion or immersion in 10% formal-saline, dissected free from the sclera and choroid, mounted ventricular (or receptor) layer up, and stained with cresyl violet. Profiles in the early (metaphase), middle (anaphase and telophase), and late (daughter cell) stages of mitosis could be distinguished. Actively mitotic cells were found over the entire ventricular surface of an E46 retina, at a density of approximately 1-2000 cells/mm^2. At E50 a small region of low mitotic activity is apparent, located 2-3 optic disc diameters superior and temporal to the disc, at the site of the developing area centralis. At this age the density of actively mitotic cells is less than 200 cells/mm^2 at the area centralis, but elsewhere in the retina is relatively uniform at about 2000 cells/mm^2. At subsequent ages the region of low (eventually zero) mitotic activity extends to include about 60% of the retina at birth where mitotic activity is confined to the peripheral margin of the retina. In a P10 retina no mitotic activity was detected, even at the most peripheral edge of the retina.

These initial findings confirm previous reports that in the neonatal cat retina mitotic activity is confined to the peripheral margin of the retina. They show this neonatal pattern to be a late stage in a patterned decrease of mitotic activity. The decrease commences at about E50, at the region of the area centralis; it therefore begins at the same time and location as the initial formation of the outer plexiform layer (Rapaport,D.H. and Stone,J., Dev. Brain Res., in press).

236.1 AN IDENTIFIED HISTAMINERGIC NEURON MODULATES LIP AND MOUTH FEEDING MOVEMENTS IN APLYSIA. Chiel, H.J., Weiss, K.R., and Kupfermann, I. Center for Neurobiology and Behavior, Columbia Univ. New York, N.Y. 10032

The identified histaminergic neuron of Aplysia, C2, (Weinreich and McCaman) has extensive synaptic actions on cells in the cerebral ganglion, including the metacerebral cell (MCC), in which it produces a slow decreased conductance EPSP. Since the MCC modulates feeding behavior, we studied the possible function of C2 by examining its actions, and those of its followers, in a reduced preparation consisting of the head ganglia connected to the tentacles, lips, jaws, and the extrinsic buccal muscles. Firing of C2 does not produce any short latency motor responses, although prolonged firing can produce lip movements of variable magnitude and latency. C2 has two readily identifiable excitatory followers, C4 and C5, and a large number of inhibitory followers in the E cluster. C4, the largest cell in the E cluster, receives a slow decreased conductance EPSP from C2 similar to that which C2 produces in the MCC. C5, the other excitatory follower, and C6, an inhibitory follower, both receive increased conductance PSPs from C2. Although C4 and C5 have axons in the periphery, firing them produces no short latency motor responses. Several observations indicate that C2 may exert widespread modulatory effects on feeding-related behaviors. The inhibitory follower C6 has a powerful, short latency motor effect: it serves to bring the lips together, in a manner that appears similar to the lip closing response in an intact animal when it grasps a piece of seaweed. Activity of C4 suppresses movements induced by firing the inhibitory follower C6. Furthermore, in a semi-intact preparation, C4 and C5 receive bursts of synaptic activity which are in phase with buccal, lip,and jaw movements, and are transmitted via the cerebral-buccal connectives (CBC). Firing of C2 reduces the size of individual EPSP's that reach both cells during these bursts. Since C2 produces a postsynaptic increase in the input resistance of C4, which would ordinarily function to increase the size of synaptic input, the reduction of the EPSP's in C4 may be due to presynaptic inhibition. We obtained further support for this hypothesis by bathing the cerebral ganglion in a high Mg^{2+}, high Ca^{2+} solution, which blocks polysynaptic activity, and stimulating the CBC, which produces an EPSP in C4. Firing C2 simultaneously decreased the EPSP and increased the input resistance of C4. Firing of the histamine cell may thus "gate" inputs to C4, enhancing some postsynaptically, while simultaneously inhibiting others presynaptically. Our results suggest that the histamine cell operates on several levels to modulate lip and mouth movements during feeding. Possible afferent functions of this cell (Weiss, Chiel, Kupfermann, and Koch) support this notion.

236.2 MONOAMINES AND THE CONTROL OF THE FEEDING SYSTEM IN LIMAX MAXIMUS. S. J. Wieland and A. Gelperin. Dept. of Biology, Princeton Univ. Princeton, N.J. 08544 and Bell Labs., Murray Hill, N.J. 07974.

Limax maximus is capable of rapid, robust associative learning as an intact animal (Gelperin, A., Science 189: 567-570, 1975; Sahley, C. et al., Proc. Natl. Acad. Sci. 78: 640-642, 1981) and as a radically dissected nervous system plus lip chemoreceptive structures (Chang and Gelperin, Proc. Natl. Acad. Sci. 77: 6204-6206, 1980). Learned behavior in the isolated nervous system is expressed through selective suppression of the feeding response to aversively-conditioned stimuli. To approach a cellular basis for feeding control and associative learning, we are delineating the neurons in the cerebral ganglion which receive sensory input, and those which project to the buccal motor ganglion to initiate, modulate or suppress expression of the feeding motor program.

Retrograde labelling of axons located approximately 15 cerebral neurons which project to the buccal ganglion and are candidates for feeding-control interneurons. Of these, 6 lie in clusters which contain dopaminergic cells and one, the metacerebral giant cell (MGC), is known to contain serotonin. We then examined the distribution and physiology of these monoamines in the cerebral and buccal ganglia. Using HPLC with electrochemical detection, the cerebral ganglia were found to contain 380 pmol serotonin and 120 pmol dopamine per animal; the buccal ganglia contained 30 pmol serotonin and 19 pmol dopamine. No norepinephrine nor oxidized metabolites of monoamines were detected. Using ^{3}H-tyrosine as a precursor, we detected accumulation of ^{3}H-dopamine, but little, if any, ^{3}H-norepinephrine nor ^{3}H-octopamine in both ganglia.

Exogenous serotonin flowing over the nervous system produced general excitation of neurons with axons in the buccal motor roots. High concentrations ($>10^{-5}$M) produced excitation with synchronized bursting, but seldom conventional feeding motor program. These results are consistent with the role of the MGC as a positive modulator, but not as a trigger of the feeding response. In contrast, dopamine selectively affected buccal motor root activity at lower concentrations (10^{-7} to $3x10^{-6}$M). The interburst interval of the autoactive salivary fast burster cell decreased in a dose-dependent manner, while the slow salivary bursters were inhibited; extracellular monitoring revealed no modulation of other units. At higher concentrations ($3x10^{-6}$ to $3x10^{-5}$M) feeding was triggered in a dose-dependent manner. The probability of triggering feeding was: 17% at $3x10^{-6}$M, 33% at 10^{-5}M, and 100% at $3x10^{-5}$M. Dopamine is thus a strong modulator of the feeding system and is a good candidate transmitter for the endogenous trigger of the feeding response. Supported by NIH Grant NSMH 15698 and NSF Grant BNS 8005822.

236.3 APPETITIVELY REINFORCED ODOR-CONDITIONING MODULATES FEEDING IN LIMAX MAXIMUS. C.L. SAHLEY, P. Hardison*, A. Hsuan* and A. Gelperin. Dept. of Psychology, Yale University, New Haven CT, 06520, Dept. of Biology, Princeton University, Princeton, NJ 08544 and Bell Laboratories, Murray Hill, NJ 07947.

Feeding behavior in Limax consists of two phases, a foraging phase primarily guided by odor cues and an ingestive phase primarily triggered by taste cues. Similar components of feeding behavior have been described in Aplysia (Kupfermann, I., Behav. Bio. 10: 89, 1974). Previously we have demonstrated that the foraging phase can be modulated by associative learning. Locomotion toward a preferred odor, (carrot or potato) can be attenuated following the pairing of the odor with a bitter taste (Sahley, C. et al., Proc. Natl Acad. Sci. 78: 640, 1981). We report here that Limax can also learn an appetitively reinforced odor-taste association. That is, a slightly aversive odor can be made attractive following several paired presentations of the odor and fructose.

Using a Pavlovian conditioning paradigm, slugs were exposed to two odor stimuli, methylcyclohexanol (MCH) and amyl acetate (AA), and one taste stimulus (fructose). Slugs in the experimental group experienced a direct pairing of the MCH and fructose. Slugs in the control group received explicitly unpaired presentations of MCH and fructose. Twenty-four hours later, odor preferences for all slugs were evaluated in a MCH versus AA odor choice test by measuring the amount of time spent over each odor within 3 one minute trials. After training, the slugs in the paired group readily approached and spent time over the MCH. In contrast, slugs in the unpaired group continued to avoid the MCH as do naive slugs. This association is acquired within 10 trials and is retained up to 12 days.

These odor-taste associations which influence Limax's foraging also modulate the slugs ingestive behavior. Initial observations had indicated that food-odors can elicit biting on plain agar disks in food deprived slugs; whereas non-food odors, including MCH, do not. Recent experiments indicate that for slugs which have experienced MCH-fructose pairings, MCH has acquired the ability to elicit feeding. MCH triggered feeding was not observed for slugs in the control group. However, control slugs did feed on the agar in the presence of carrot-odor.

Thus, both phases of Limax's feeding behavior, foraging and ingestive, can be modulated by a single learned odor-taste association. From this behavior we conclude that the neural change underlying the odor-taste association has access to and influences two distinct motor systems.

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236.4 IN VITRO EXPRESSION OF IN VIVO LEARNING BY THE CEREBRAL GANGLIA OF THE TERRESTRIAL MOLLUSC LIMAX MAXIMUS. A. Gelperin and N. Culligan*. Dept. of Biology, Princeton Univ., Princeton, N. J. 08544 and Bell Labs., Murray Hill, N. J. 07974

The giant garden slug Limax maximus can learn in one trial to associate new odors and tastes with toxicosis (Gelperin, A., Science 189: 567, 1975; Sahley, C. et al., Proc. Natl. Acad. Sci. 78: 640, 1981). After learning has occurred, an olfactory stimulus which previously caused slugs to approach the stimulus source now causes locomotor avoidance or withdrawal. Other experiments have demonstrated that a preparation of the lips, cerebral ganglia and buccal ganglia (LCB) can also show one-trial learning (Chang, J. & Gelperin, A., Proc. Natl. Acad. Sci. 77: 10, 1980; Gelperin, A. & Culligan, N., in preparation, 1982). The LCB preparation learns to suppress feeding motor program (FMP) normally triggered by application of standardized food extracts to the lips when lip stimulation by a food extract is paired with lip stimulation by bitter plant substances such as quinidine. The experiments reported here examined the link between the whole animal learning and the plasticity of the LCB preparation.

Intact slugs were given 4-7 training trials during which the application of an attractive food extract (CS) was paired with the application of quinidine (US). Interspersed with the learning trials, slugs were given exposures to a second attractive food extract (S2) whose application was not paired with quinidine. Two or three days after the last training trial, slugs were dissected into an LCB preparation arrayed so that the CS and S2 food extracts could be applied to the isolated lips while buccal nerve root recordings monitored the generation of FMP. Of 14 slugs trained as intact animals and tested as LCB preparations, 7 (50%) showed a clear and dramatic differential FMP response to the CS and S2 food extracts, i.e., on the majority of CS test trials no FMP response occurred while the S2 test trials 30 min. before produced clear bouts of FMP. A control group of 4 slugs received the same intact animal training paradigm as the experimental group except that the CS and US were temporally unpaired, i.e., the US followed 30 minutes after the CS application. All of these slugs showed clear FMP responses to both the CS and S2.

We conclude that the food-odor preference changes produced in the whole animal by associative learning and the changes in chemosensory input-FMP output relations produced in the isolated LCB preparation by associative learning result from neuronal events at some shared central locus of synaptic plasticity. (Supported by NIH Grant NSMN 15698 and NSF Grant BNS 8005822.)

236.5 POSTURAL LEARNING IN 'WETAS' IS MEDIATED BY OCTOPAMINE-MODULATED NEUROMUSCULAR EVENTS AND 'CATCH' CONTRACTION. Graham Hoyle. Dept. of Biology. Univ. of Oregon, Eugene, OR. 97403. Carried out during sabbatical leave at Canterbury Univ., Christchurch, N. Z. in the lab of Dr. L. H. Field.

The ancient orthopteran insects known as 'Wetas' have, in New Zealand, been separated from the stem that led to locusts and cockroaches for some 10^8 years. A common species, Hemideina femorata, was trained by operant-conditioning using a low-frequency sound as negative reinforcement. The trained metathoracic tibia occupied a position-window, pre-set in either extension or flexion, away from rest position. The window was occupied abruptly, within about 60 seconds, following a series of quick flex-extend-flex movements through the window. During occupation of the window, which was maximally 2 h. 46 min. before the first 'error', no electromyographic activity occurred regardless of window position. A pair of very smooth apposed cuticular plates in the joint press against each other and provide a force which restores the femero-tibial resting angle of 76°. A sustained extensor effort of 7 g increased the angle to 135°; flexor effort of 5 g reduced it to about 30°. The relevant muscles were found to produce such tensions, following a brief burst of slow-axon excitation, only occasionally in untreated animals, but consistently following either a brief burst in the octopaminergic neuron DUMETi, or infusion of 10^{-8} M octopamine.

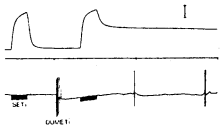

Figure: extensor tibiae isometric tension response (top trace) to slow extensor (SETi) excitation at 8 Hz; first before, then after, an electrically-driven burst in DUMETi. The second mechanical response is facilitated, and also has a 'catch'-like remainder

The octopamine-mediated 'catch'-like contraction is associated with a maintained depolarization in many, but not all, muscle cells, following neural excitation. Repolarization occurs very slowly spontaneously, after a delay of several minutes, but rapidly following a single inhibitory impulse or late excitatory one. Leg-position learning using this peripheral 'catch' mechanism, and its unlocking by an inhibitory axon, may represent the primitive mode of learned postural adjustment. It appears to be made possible by suppression of post-synaptic repolarizing conductance by octopamine, and rapid de-suppression by synaptic activation of repolarizing electrogenesis. This mechanism is absent, or greatly reduced, in locusts and cockroaches. Supported by NSF Research Grant BNS 82-41884.

236.6 CONVERGENCE OF VISUAL AND STATOCYST INPUTS ON INTERNEURONS AND MOTORNEURONS OF HERMISSENDA: A NETWORK DESIGN FOR ASSOCIATIVE CONDITIONING. Y. Goh* and D.L. Alkon. Section on Neural Systems, Lab. of Biophysics, NINCDS, NIH, MBL, Woods Hole, MA 02543

To assess directly the behavioral significance of neuronal changes during learning, a comprehensive description of relevant neural systems is necessary (Alkon, Biol. Bull. 159, 505, 1980). In this report we have investigated the convergence of visual and statocyst input on interneurons which directly excite MN1 cells, paired identified neurons, one in each of the two pedal ganglia. MN1 impulse trains elicited by positive current pulses cause ipsilateral turning of the posterior half of the animal's foot. MN1 impulse frequency also increases in response to illumination of the animal's eyes and thus should play a role in the animal's movement toward areas of maximal light intensity. MN1 impulse frequency increases 0.5-1.0 sec after light onset and undergoes a delayed increase (15-70 sec) with a rhythmic bursting pattern. Impulses of both the medial Type A photoreceptor and statocyst hair cells are followed one-for-one with a brief latency (5-7, 6-9.5 msec, respectively) by EPSPs recorded from identified cerebropleural interneurons (IN cells). Impulses of these IN cells in turn are followed one-for-one with brief latency (8-10 msec) by EPSPs recorded from the MN1 cell. Thus, medial Type A impulses elicited by light or a positive current pulse produce indirectly EPSPs and increased impulses of MN1 cells by directly exciting IN cells. Impulses of specific hair cells produce the same indirect excitation of MN1 cells. The anatomic basis of this intersensory convergence was established by intracellular injection of Lucifer Yellow into pairs of neurons which showed the synaptic relations just described. Photoreceptors and hair cells terminate (∿ 80 μm from the somata) on each other as well as on endings of the IN cell whose somata are located within the cerebropleural ganglion 60-70 μm from the sensory cells. IN cells send axons to terminate within the pedal ganglia on endings of the MN1 cell which sends its axon through an exiting pedal ganglial nerve coursing to the foot. Type B impulses inhibit the medial Type A cell and thus ultimately MN1 responses to light. Because pairing of light and rotation (but not unpaired stimuli) enhances Type B depolarizing responses (and thus increased impulse activity) to light, MN1 cells should show decreased responsiveness to light stimuli in associatively conditioned animals. This and other predictions based on this network have been confirmed. The neural network consisting of peripheral sensory interactions between photoreceptors, hair cells and optic ganglion cells and the visual-statocyst convergence on IN and MN1 cells, therefore, provides a basis for Hermissenda associative conditioning and possibly can serve as a model for conditioning networks in other animals.

236.7 TYPE B PHOTORECEPTOR CHANGES PREDICT MODIFICATION OF MOTORNEURON RESPONSES TO LIGHT DURING RETENTION OF HERMISSENDA ASSOCIATIVE CONDITIONING. I. Lederhendler*, Y. Goh* and D.L. Alkon (SPON: G. Acheson). Sect. Neural Systems, Lab. Biophys., NINCDS, NIH, MBL, Woods Hole, MA 02543.

The results of several previous studies have indicated a causal role for long-term membrane changes of Type B photoreceptors in producing associatively learned behavior of the nudibranch Hermissenda crassicornis. Changes of an early rapidly inactivating voltage-dependent K^+ current, I_A, could explain enhanced sustained responses of Type B cells during and following light steps on retention days. These changes were identified for Type B somata which had been isolated by axotomy from all synaptic interactions and thus they were intrinsic to the Type B cell itself and not a consequence of other pre-synaptic changes. Type B impulses inhibit medial Type A impulse activity and thereby reduce excitation in response to light of interneurons and putative motorneurons, MN1 cells, which can control turning movements in response to light. Furthermore, caudal hair cell impulses elicited by current injection (or S/E optic ganglion cell depolarization elicited by current injection) when paired repeatedly with Type B impulses elicited by light cause the same neural changes as observed for associatively conditioned animals (Farley and Alkon, Soc. Neurosci. Abstr., 1982). To further test the causal role of Type B changes in associative learning we examined Type B input resistance, which is significantly determined by I_A (Shoukimas and Alkon, in prep.), in relation to MN1 impulse activity in response to light (MN1-LR). Type B input resistance, significantly elevated on retention days, was inversely correlated with MN1-LR (r_S=-.46, P<.005). This correlation was predicted by the previous observations that (1) the Type B response to light is enhanced by conditioning, and (2) Type B impulses indirectly inhibit (see above) MN1-LR. Also as expected, MN1-LR were lower for conditioned animals during retention when compared to naive (t=2.96, P<.02) as well as unpaired control groups (t=2.75, P<.02). Furthermore, particularly for associatively conditioned animals, MN1-LR was positively correlated with the degree of reduction of positive phototactic behavior of individuals (r_S=.52, P<.05). The latter and other observations indicate a close relationship of MN1-LR to the ability of animals to turn toward areas of maximal light intensity. The data presented here lend further support to the proposal that membrane changes intrinsic to the Type B cell are expressed by associative learning behavior of intact animals.

236.8 SENSORY NEURONAL CORRELATES OF ASSOCIATIVE LEARNING IN HERMISSENDA. T. Crow, Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Hermissenda exhibits a modification of visually guided locomotor behavior that is produced by a conditioning procedure. The conditioning procedure involves the temporal pairing of light with a gravitational stimulus (rotation) (Crow and Alkon, 1978) which results in long-term changes in a number of light dependent measures of phototaxic behavior including the initiation of locomotion during illumination and avoidance of some light intensities (Crow and Offenbach, 1979). In this report neural correlates of the conditioning procedure related to changes in visual sensitivity are examined in the photoreceptors in order to study the role adaptation may play in contributing to the features of the behavior.

Animals receiving paired presentations of light and rotation showed significant increases in the time taken to initiate locomotor behavior in the presence of light and significant decreases in the amount of time spent in the brighter part of a light gradient as compared with control groups receiving random presentations of light and rotation. Intracellular recordings from medial and/or central type B photoreceptors 24 and 72 h following behavioral training revealed a number of differences in measures reflecting photoreceptor adaptation between experimental and control groups. The latency of the photoresponse was significantly ($p < .05$ for all comparisons) increased for all light intensities tested for trained (N=7) subjects as compared with controls (N=6). In order to avoid secondary complications due to spike activity and synaptic input a second series of experiments were performed with axotomized photoreceptors. Again training (N=5) resulted in a significant increase ($p < .01$ for all comparisons) in the latency of the photoresponse as compared with random control groups (N=8). The differences in the latency of the photoresponse between trained and random controls was largest 24 h after training and decreased when tested 72 h after training. Since the latency of the photoresponse is a concomitant of changes in visual sensitivity, studies were conducted to examine photoreceptor desensitization. Results from preliminary studies indicate that B photoreceptors from trained animals exhibited less light induced desensitization than random control groups.

Changes in the photoresponse to light may reflect alterations of intracellular Ca^{++} concentration as has been suggested for light adaptation in Limulus ventral photoreceptors. Thus changes intrinsic to the photoreceptors such as Ca^{++} regulated receptor sensitivity could make a contribution to the light response of the photoreceptors and may help explain the associative behavioral changes.

236.9 K^+ CONDUCTANCES AND PROTEIN PHOSPHORYLATION IN HERMISSENDA: DECREASE IN ^{32}P INCORPORATION IN A 24,000 MW PHOSPHOPROTEIN BAND IN THE PRESENCE OF 4-AMINOPYRIDINE. J.T. Neary and D.L. Alkon, Section on Neural Systems, Lab. of Biophysics, NINCDS, Marine Biological Lab., Woods Hole, MA 02543.

Recent interest in the modulation of membrane conductances, particularly K^+ conductances, has focused on biochemical mechanisms such as protein phosphorylation. In this abstract, we present evidence which suggests that protein phosphorylation-dephosphorylation is related to the fast outward K^+ current, I_A, in neurons from the nudibranch mollusc, Hermissenda crassicornis.

K^+ currents can be separated pharmacologically: in Hermissenda photoreceptors and other neurons, 4-aminopyridine (4-AP) preferentially blocks I_A while Ba^{2+} preferentially blocks a delayed outward K^+ current, I_B (Alkon et al., Biophys. J., in press, 1982). In order to test the effects of these agents on protein phosphorylation-dephosphorylation systems in Hermissenda, circumesophageal nervous systems were incubated in $^{32}PO_4^{2-}$ to label phosphoproteins, rinsed in artificial sea water (ASW), and transfered to ASW solutions containing the pharmacological agent of interest. Tissues were then placed on ice and eyes and pedal ganglia were dissected and homogenized. ^{32}P-phosphoproteins were separated by SDS-polyacrylamide gel electrophoresis and detected by autoradiography and densitometry. Apparent molecular weights (MW) are given below.

We found that incubation in 4-AP results in a 7-10 fold decrease in ^{32}P incorporation in a 24,000 MW phosphoprotein band (24K PP) in eyes, pedal ganglia, and circumesophageal nervous systems, whereas Ba^{2+} has a much smaller effect on the level of incorporation in 24K PP. Depolarization of neurons with 100 mM external K^+ has an effect similar to that of 4-AP. The decrease in 24K PP is dependent on the time of incubation and the concentration of 4-AP with maximal effect observed at 20-25 min and 10 mM 4-AP. The effect is partially reversible, i.e. 24K PP reappears when 4-AP is removed. Preliminary experiments indicate that during recovery from 4-AP blockade, ^{32}P incorporation is increased in a Ca^{++}-dependent phosphoprotein band (22,000 MW). The expression of the 4-AP effect appears to require intact neurons since addition of 4-AP to a broken cell preparation did not affect 24K PP.

These studies suggest a correlation between I_A and protein phosphorylation-dephosphorylation. They provide a biochemical demonstration of a difference in two types of K^+ currents, I_A and I_B, since 4-AP, an I_A blocker, dramatically reduced 24K PP while Ba^{2+}, an I_B blocker, had a much smaller effect on the same band. This finding may be related to the observation that I_A, but not I_B, is reduced in photoreceptors of animals previously conditioned with paired (but not unpaired) light and rotation (Alkon et al., Science 215, 693, 1982).

236.10 Ca^{2+}-DEPENDENT PROTEIN KINASE REGULATION OF $K^+(V)$-CURRENTS: A POSSIBLE BIOCHEMICAL STEP IN ASSOCIATIVE LEARNING OF HERMISSENDA. J. Acosta-Urquidi, J.T. Neary, and D.L. Alkon. Sect. Neural Systems, Lab. Biophys., NINCDS, NIH, MBL, Woods Hole, MA 02543

Primary membrane changes (viz. a reduced early transient $I_K+(V)$, I_A, and enhanced long-lasting depolarization, LLD, following a light step) in isolated (axotomized) Type B photoreceptors were observed to be retained on days following associative conditioning of intact nudibranch molluscs (Hermissenda c.) with paired light and rotation, but not randomized stimuli (Alkon et al., Science 215, 693, 1982). During the acquisition period, changes in the levels of phosphoprotein bands have also been detected in the eyes (Neary et al., Nature 293, 658, 1981). To probe possible biochemical mechanisms of conditioning, in the present study, we iontophoretically injected (0.6-1.0 nA, for 1 min) into isolated Type B somata a Ca^{2+}-calmodulin activated protein kinase, phosphorylase kinase (PhK). Injection of PhK (0.2 mg/ml) had no significant effect on dark input resistance (R_i), but significantly increased R_i (42.6%, P<.01) only after presentation of one or more 30 sec light steps. No significant increase in R_i followed PhK injection and light steps in Ca^{2+}-free SW (t=0.39, d.f. 11), indicating that Ca^{2+} influx is required for this effect. In normal Ca^{2+}-SW FhK injection also enhanced LLD (640%, P<.01), which results in part from a light-induced $I_{Ca}(V)$. Under voltage-clamp, PhK injection (in normal Ca^{2+}-SW) significantly reduced I_A (20.37% P<.001), only after the presentation of one or more 30 sec light steps paired with a command step (+50-60 mV). These findings are of interest in view of the reported Ca^{2+}-dependent inactivation of I_A in Type B cells after conditioning (Alkon et al., Biophys. J., in press, 1982). Identical iontophoretic injections of a control carrier solution (0.95 KAc, .05 mM TRIS, pH 8.6) were without significant effect on R_i, LLD or I_A and I_B. By contrast, iontophoretic injection of the catalytic subunit of cAMP-dependent protein kinase (PKC) caused a significant increase in dark R_i and LLD (Acosta-Urquidi et al., Soc. Neurosci. Abstr. 7, 944, 1981). Under voltage-clamp, PKC injection reduced I_B (46%, P<.001) to a greater extent than I_A (20%, P<.025). Based on these and other data, a causal sequence during conditioning of Hermissenda might be as follows: paired light and rotation cause (via interactions of optic and statocyst pathways) enhanced LLD and cumulative depolarization of Type B cells; elevation of $[Ca_i^{2+}]$ (Connor and Alkon, Soc. Neurosci., 1982); activation of Ca^{2+}-CAM protein kinase(s); changes in the phosphorylation levels of protein(s); decreased I_A; increased LLD on retention days, decreased motor-neuron output (Goh et al., Soc. Neurosci., 1982) and decreased phototactic response.

236.11 CUMULATIVE CELLULAR DEPOLARIZATION AND SHORT TERM ASSOCIATIVE CONDITIONING IN HERMISSENDA. J. Farley* and D.L. Alkon. (SPON: J. Atema). Dept. Psychol., Princeton University, Princeton, NJ 08540, and Sect. Neural Systems, NINCDS, NIH, at Marine Biol. Lab., Woods Hole, MA 02543

Type B photoreceptors in the eyes of Hermissenda have previously been shown to exhibit both short- (Crow and Alkon, Science 209, 412, 1980) and long-term (Farley and Alkon, J. Neurophysiol. in press, 1982) increases in input resistance, and enhanced light-induced depolarization and impulse activity, following the exposure of specimens to repeated pairings of light and rotation. These electrophysiological changes in B cells are believed to represent a neural substrate for retention of associative modifications of phototaxis in this animal. Here, we describe a relatively short-term neural mechanism for acquisition of the long-term changes: cumulative depolarization of Type B photoreceptors.

Our study of cumulative depolarization has been aided by the development of an in vitro method for simulating the associative training received by intact animals. This simulation entails stimulating and recording from three clases of neurons in the isolated nervous system, normally affected by the associative training procedure for intact animals: a Type B photoreceptor, the S/E optic ganglion cell, and a caudal hair cell in the statocyst. Exposure of isolated n.s. preparations to five pairings of light and current-induced impulse activity in the caudal hair cell produced an average 10 mV depolarization of the Type B cell, significantly greater (P<.001) than that occurring for five random presentations of these two stimuli. Determining features of this visual-statocyst network which give rise to cumulative depolarization are: (a) the long-lasting depolarization (LLD) response of the B cell to light, (b) two distinct varieties of synaptic facilitation of the LLD response. Following each pairing, we observed increased EPSP frequency and magnitude in Type B cells, and a disinhibition of these same cells due to a post-stimulation hyperpolarization of the caudal hair cell. Pairing-produced depolarization of the Type B cell was significantly reduced (P<.001) when the S/E cell - the source of the EPSPs - was hyperpolarized throughout the course of pairings, and also when the disinhibition of the Type B cell by the caudal hair cell was precluded. Conversely, pairings of light and depolarizing current injections to the S/E cell were sufficient to produce prolonged and pairing-specific depolarization of the B cell. Finally, exposure of intact animals to five pairings of light and rotation produced short-term, pairing-specific (P<.05), phototaxic suppression, which paralleled the duration of B cell depolarization.

These and other results implicate cumulative depolarization and concomitant elevated intracellular Ca^{++} in producing long-term inactivation of I_A, a voltage-dependent K^+ current (cf. Alkon et al. Biophys. J., in press, 1982).

237.1 BLOCK OF AXOPLASMIC TRANSPORT BY AGENTS INTERFERING WITH CALCIUM FLUX: COBALT, NICKEL, LANTHANUM, VERAPAMIL; AND MAINTENANCE OF TRANSPORT IN CALCIUM-FREE MEDIA BY STRONTIUM. S. Ochs. Dept. of Physiology and Biophysics Prog., Indiana Univ. School of Med., Indianapolis, IN 46223.

Using the desheathed cat peroneal nerve preparation, Ca was shown to be required to maintain axoplasmic transport (Ochs, et al, Nature, 270: 748, 1977). We would expect that agents blocking the entry of Ca into the axons would also be effective in blocking axoplasmic transport. For these experiments we injected cat L7 dorsal root ganglia with ^{3}H-leucine in accordance with our usual procedure, allowing 2 hr of axoplasmic transport to carry labeled proteins for a distance down the peroneal and tibial branches of the sciatic nerve. The nerves were removed, a long length of the peroneal branch desheathed, and the preparation placed in a given medium for a further 3-6 hr of in vitro downflow. With Ca present, concentrations of 1-30 mM Co effected a block. And, concentrations of 1.5-10 mM verapamil also blocked. 2.5-10 mM Ni was not quite as effective. Some degree of competitive interaction between Ca and Co was observed, with 20 mM Ca causing a small decrease in the blocking effect of 5 mM Co. Such a competing interaction of Ca with verapamil was not seen. Conversely, some ions can substitute for Ca in maintaining transport. In our earlier studies (Chan, et al, J. Physiol., 301: 477, 1980) we found Mg was only partially effective in this respect, and high concentrations of 50-100 mM K able to support transport fairly well. Ba and Sr have been reported to substitute for Ca in maintaining some membrane properties. In the present study, Sr was found able to maintain transport when added in concentrations of 1.5 to 30 mM to a Ca-free medium. Ba when present in concentrations of 5-10 mM was not able to do so. We may interpret the results in accord with our present concept of the transport mechanism (Ochs, Neurosci. Res. Prog. Bull., 20: 19, 1981). In the transport-filament model, a micromolar level of free Ca is required in the axon to activate calmodulin and in turn Ca-Mg ATPase which utilizes the ~P of ATP to drive the transport filaments and in turn, the various materials bound to them. Removal of Ca from the medium, or blocking the entry of Ca by Co, Ni, La or verapamil while a Ca-Na exchange or a Ca pump in the membrane continues to operate, would deplete Ca in the axon to the point where calmodulin would be inactivated. Sr could maintain transport by decreasing the efflux of Ca, retaining it in the axon. Alternatively, Sr could enter the axon and substitute for Ca in activating calmodulin, an hypothesis at present under investigation. Supported by NIH R01 NS 8706-13 and NSF BNS-7914029.

237.2 ISOLATION OF AXONALLY TRANSPORTED GLYCOPROTEINS WITH MYELIN OF INTACT AND DEGENERATING OPTIC TRACT. J. Elam and L. Elmer*, Dept. Bio. Sci., Florida State Univ., Tallahassee, FL 32306

A previous study in our laboratory showed the association of axonally transported ^{3}H fucose labeled glycoproteins with goldfish optic tectal myelin (Monticone & Elam Br Res 100 61 1975). SDS PAGE of transported glycoproteins in myelin showed a concentration of label in glycoproteins of approximately 50 K daltons. To further assess the specificity of axonal transport labeling of myelin, these studies have now been extended to the anatomically less complex optic tract.

Tract myelin analyzed three days after glial incorporation of intracranially injected fucose showed labeling of a variety of high molecular weight glycoproteins as well as a distinct peak of radioactivity comigrating with proteolipid protein. Tract myelin isolated 3-10 days after intraocular injection of fucose showed a clearly different labeling pattern with a concentration of radioactivity near 54 K daltons, secondary peaks at 45 and 70 K daltons and no label migrating with proteolipid. Some degree of specificity in the glycoproteins co-isolated with myelin was indicated by the absence of a similar labeling pattern in higher density membrane of the tract.

As a further test of the specificity of glycoproteins co-isolating with myelin, analysis of transport labeling was conducted in tracts undergoing Wallerian degeneration. This study addressed the hypothesis that axonal glycoproteins closely affiliated with myelin would turn over in parallel to the breakdown of myelin rather than in parallel to the breakdown of the axon. Results showed that 9 days after enucleation, degenerating tracts retain 60% of their original myelin and only 23% of previously transported ^{3}H fucose. An increased proportion (50%) of the surviving radioactivity co-isolated with myelin and SDS PAGE analysis showed a marked concentration of the surviving label in the glycoprotein of 54 K daltons. There was no similar concentration of this glycoprotein in other membranes of the degenerating tract.

Taken together, the results support the view that certain specific axonal glycoproteins co-isolate with myelin, possibly as constituents of paranodal junctions. At least one of these glycoproteins turns over in parallel to myelin during Wallerian degeneration.

237.3 COMPARATIVE COMPOSITIONAL ANALYSIS OF SLOWLY TRANSPORTED AXONAL PROTEINS IN PERIPHERAL AND CENTRAL MAMMALIAN NEURONS. M. Oblinger, S.T. Brady, and I. G. McQuarrie. Department of Anatomy, Case Western Reserve School of Medicine, Cleveland, Ohio 44106.

Different axonal systems vary in their morphology, function and regenerative capacity while the rate and composition of the fast component of axonal transport is relatively constant from one type of neuron to another. However, the rates of the two slowest components of axonal transport, slow component a (SCa) and b (SCb), both characterized by distinct constellations of major polypeptides (Tytell et al., 1981, Science, 214:179-181), differ markedly between different populations of nerve cells (McQuarrie et al., 1980, Soc. Neurosci. Abst., 6:501). Previous observations from this laboratory indicating differences in the rates of slow transport and some compositional variations in SCa and SCb in different types of mammalian neurons prompted a careful compositional analysis of the two slowest rate components of axonal transport in three different axonal systems of the rat. Two peripheral systems, the sensory and motor axons of the sciatic nerve, which successfully regenerate and a central sensory system, the optic nerve, which fails to regenerate, were chosen for qualitative and quantitative comparisons of polypeptides conveyed in SCa and SCb in intact adult axons of the rat.

^{35}S-methionine was microinjected into either the motor horn of the L3-L5 spinal cord, the L5 dorsal root ganglion, or the intraocular cavity and the sciatic or optic nerves retrieved after periods of time (6-60 days) appropriate to allow separation of the SCa and SCb peaks in the axons. The nerves were cut into 2mm segments and segments containing peak radioactivity associated with the two rate components subjected to high resolution 2D-PAGE and fluorography.

The 2D profiles of major labelled proteins associated with SCa are qualitatively similar in the three systems examined. Among the major differences is the relatively greater amount of neurofilament triplet proteins to tubulin in peripheral motor and sensory axons compared to optic axons. The relative amounts of the putative tau proteins may also be different in these systems. The sensory and motor sciatic axons contain similar profiles of SCb proteins at peak regions and differ from optic axons in several respects: they contain considerable tubulin that extends into SCb peak regions and lower relative amounts of two metabolic enzymes, creatine phosphokinase and nerve specific enolase compared to actin. Qualitative and quantitative differences in several other proteins are present. These compositional differences in slowly transported axonal proteins in conjunction with kinetic differences may have important consequences on the physiological properties of different classes of neurons, such as the ability to successfully regenerate.

237.4 ISOLATION OF AXONALLY TRANSPORTED PARTICLES FROM ADRENERGIC NERVE. D. Studelska*, S. Brimijoin, Mayo Clinic, Rochester, MN 55905.

Rapid anterograde axonal transport of dopamine-β-hydroxylase (DBH) is thought to reflect movement of transmitter-storage vesicles in adrenergic axons. However, since it appears to lack norepinephrine (NE), the particle responsible for retrograde transport of DBH is unknown. It is also uncertain what particle is involved in retrograde axonal transport of nerve growth factor (NGF). For a direct study of these transported particles we undertook to isolate them from peripheral nerve.

The sciatic nerves of Sprague-Dawley male rats (200 g) were ligated in situ. In some cases ^{125}I-NGF was injected into the hind footpads. After 16 hr, 3-mm samples were taken from above or below the ligatures (to collect anterogradely and retrogradely transported material, respectively). To minimize damage to organelles during homogenization, the samples were desheathed and incubated in 0.9% NaCl with collagenase (8 units/ml, 30 min, 37°C); they were then homogenized in isotonic sucrose and spun at 8000 g for 10 min. Portions of the supernatants were layered on 5 ml sucrose density gradients (.3-1.2 M), centrifuged at 150,000 g for 2 hr, and divided into 27 fractions for analysis of DBH activity, NE content and NGF label.

The homogenization conditions appear to be critical for the isolation of intact vesicles. Most procedures (i.e., use of a glass homogenizer with tightly-fitting pestle run at high speed) gave gradients with 1) broad peaks of DBH activity and labeled NGF centered on fraction 9; 2) much DBH activity near the top; 3) all of the NE in the upper layer. The fast-sedimenting DBH activity might have represented storage granules that had lost their NE, since peak size was reduced by freezing and thawing of homogenates in hypotonic buffer. Some of the fast-sedimenting DBH could also have been associated with dense membrane-fragments.

A search for better homogenization conditions showed that loose-fitting glass homogenizers with Teflon pestles run at low speed yielded a significant amount of NE that sedimented into the region of the DBH-containing peak. The position of the enzyme peaks was the same regardless of whether the sample was from a proximal or a distal accumulation. This suggested that the particles involved in anterograde and retrograde transport of DBH are similar in size and density. In the gradients with distal nerve samples, the peak of DBH activity coincided with that of NGF label. This result could imply that transport of NGF in adrenergic fibers involves the DBH-containing particle. However, other particles must account for most of the NGF transport in sciatic nerve because distal accumulation of labeled NGF was reduced by only one-third after guanethidine-sympathectomy. (Supported in part by NIH grant NS 11855.)

237.5 FAST AXONAL TRANSPORT IN PERMEABILIZED LOBSTER GIANT AXONS. D.S. Forman, K.J. Brown* and D.R. Livengood. Dept. of Anatomy, USUHS and Dept. of Physiology, AFFRI, Bethesda, MD 20814.

We have developed a method for reactivating fast axonal transport in detergent-permeabilized giant axons from lobster. This system can be used to study the effects of probes that cannot cross the plasma membrane of living axons. Single motor axons from the walking legs are isolated and mounted on a perfusion slide and observed by light microscopy using Nomarski optics. Fast axonal transport is seen inside these axons as the saltatory movements of vesicular organelles and mitochondria. Movements are recorded using a video camera and time-lapse video tape recorder. Axons are permeabilized with 0.02% saponin in a medium designed to maintain axoplasmic function (modified from Abercrombie et al., J. Gen. Physiol., 78:413, 1981). During saponin treatment in medium without ATP, movement suddenly decreases after 5-10 min and then ceases entirely by 15 min. Mitochondria develop bead-like swellings and often fragment. At this point the axon appears to have become irreversibly permeable, at least to small molecules in the medium. Organelles remain motionless without any Brownian movement in saponin-free medium. However, when the medium is replaced with medium containing MgATP, saltatory movements immediately reappear. The reactivated movements resemble those seen in living axons, but are less numerous. They also tend to be shorter, but translocations longer than 40 μm have been observed. Organelles move in both the retrograde and anterograde directions. Most of the saltating organelles are small spherical vesicles, but mitochondria also move. In addition to saltations, many organelles show repeated, jerky, back-and-forth movements. Both the velocity and frequency of saltations appear to depend on the ATP concentration. Some slight movement can be detected with ATP concentrations as low as 10 μM, but ATP in the range of 1-5 mM appears to be needed for maximal reactivation. Movement may continue for as long as an hour after permeabilization. However, if the medium is replaced by one without ATP, all movement stops within 2 min. Movement can then be restarted by again perfusing with medium containing ATP. Movement cannot be reactivated by AMP or by the non-hydrolyzable ATP analogue, AMP-PNP. Permeabilized axons were used to examine the effect of vanadate on fast transport. Low concentrations of vanadate reversibly inhibit the ATPase of dynein but not myosin. Before permeabilization, externally applied vanadate cannot enter the axon and has no effect on transport. After saponin treatment, movement reactivated with 1 mM ATP is markedly inhibited by 50-100 μM Na orthovanadate. Movement quickly resumes if the vanadate is washed out or reduced with norepinephrine. This result is compatible with the hypothesis that dynein or a dynein-like molecule is involved in the molecular mechanism of fast transport.

237.6 SPECTRAL ANALYSIS OF THE MOVEMENTS OF INTRAAXONAL ORGANELLES. W. S. Kendal*, R. S. Smith, and Z. J. Koles*. Depts. of Surgery & Applied Sciences in Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2G3.

The anterograde and retrograde movement of organelles within axons was analysed before and after treatment with agents known to inhibit axonal transport. Axons were isolated from sciatic nerves of *Xenopus laevis*, and organelles were detected by darkfield microscopy. The movements of organelles were recorded on motion picture film at 3 frames per second. Analysis of the film yielded estimates of the power spectrum for the positional deviations of each organelle about its steady progression along the axon. Instantaneous velocities for each organelle were also computed. Solutions of colchicine, dinitrophenol, or dimethylsulfoxide, as well as heavy water, were used to partially inhibit the movement of the organelles.

In axons not exposed to inhibitors, individual organelles, which moved in either retrograde or anterograde directions, showed fluctuations in velocity about a mean value. The corresponding estimates of the power spectrum for each organelle's positional changes showed a peak power at up to 0.1 Hz.

Many organelles, whose steady transport was inhibited by any of the agents used, showed a back and forth movement with a frequency below 0.1 Hz. These longitudinal oscillations had root mean square amplitudes which varied from 0.2 to 5μm. Both the amplitudes and the frequencies of each oscillation were inconsistent with Brownian motion. Therefore, we propose that a metabolically driven, oscillatory force acted on the particles whose transport was inhibited. In addition, the evidence suggests that a similar oscillatory force acts on transporting organelles.

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237.7 AXONAL TRANSPORT OF BETA-ADRENERGIC RECEPTORS IN RAT SCIATIC NERVE. M.A. Zarbin*, J.M. Palacios, J.K. Wamsley and M.J. Kuhar (SPON: S. Bird). Dept. of Neuroscience, Johns Hopkins Sch. Med., Balto., MD 21205

The axonal transport of beta-receptors has been detected by in vitro light microscopic autoradiography. Rat sciatic nerves were surgically exposed and ligated for various times. After the ligation period ended, the nerves were removed and mounted on microtome chucks. Eight micron frozen tissue sections were then cut from the mounted nerves and thaw-mounted onto glass microscope slides. These slide-mounted tissue sections (SMTS) were incubated with 50 pM ^{125}I-cyanopindolol (CP) to label beta-receptors. Some SMTS were coincubated with 10 uM propranolol to generate blanks. The radioactivity in the tissue was determined by apposing a photographic film (XOMAT, Kodak) against the SMTS and then developing the film.

CP binding sites accumulated both proximal and distal to ligatures in a time dependent manner. Proximal to the ligature, the accumulation of binding sites increased linearly with time. The apparent transport rate was 19 mm/day. Distal to the ligature, binding sites accumulated linearly up to 8 hr. post ligation with an apparent transport rate equal to 10 mm/day. These calculated rates are very likely an underestimate of the true transport rate. At longer times, no further increase was detected distally. After 4 days of ligation, a time at which distal axons have degenerated, a large build-up was present proximal to the ligature; distally, binding sites were diffusely scattered. The latter data suggests that the transported beta-receptors are associated with neuronal elements.

When 2 ligatures were consecutively placed along the nerve, binding sites accumulated proximal and distal to the ligature closest to the spinal cord. At the other ligature, binding sites accumulated distally while only a small accumulation was detected proximally. These data suggests that the binding sites move by fast transport and that "turn around" occurs at points of transport disruption.

Zinterol displaced CP at the accumulated binding sites with an IC_{50} of 10 nM suggesting that these binding sites are beta-2 sites. Propranolol displaced CP with an IC_{50} of 10 nM. The transported sites exhibited stereospecificity: (+) propranolol was a weaker displacer than (+/-) propranolol.

Isoproterenol inhibited CP binding at the ligature with an IC_{50} of 1 uM. GppNHp (100 uM), a nonmetabolizable GTP analogue, inhibited the binding of isoproterenol to the sites but exerted a greater effect on the binding sites that accumulate proximal to the ligature. This result suggests that beta-receptors may undergo changes in their binding properties at different points in their life cycle.

237.8 FATE OF NEWLY SYNTHESIZED PROTEINS USING AN IN VITRO AXONAL TRANSPORT SYSTEM IN APLYSIA. P.F. Drake, M. Oblinger, and R.J. Lasek. Department of Anatomy, Case Western Reserve School of Medicine, Cleveland, Ohio 44106.

The protein synthetic machinery in the neuron is restricted to the cell body; however, not all of the neuron's newly synthesized proteins are destined for axonal transport. We have been studying the regional localization of cytoskeletal proteins in Aplysia neurons and have found that as a percent of total protein, tubulin predominates in the cell body while neurofilament proteins predominate in the axon (Drake and Lasek, 1982, Soc. for Neurochem. Abs., 13:64). If one observes the profile of newly synthesized proteins after incubation of CNS ganglia in 35S-methionine and assays the proteins from a single cell body on 2D-PAGE, it is evident that tubulin, actin and fodrin are among the major proteins; the neurofilament proteins are not among this major class.

To better understand the process of protein synthesis and transport in the neuron we have investigated the proteins of fast and slow axonal transport using an in vitro pulse-chase system. Isolated abdominal ganglia with their long pleural-abdominal (axon containing) connectives intact were excised and the ganglia pulsed in 100 μl ASW containing 2 mCi ^{35}S-methionine for 30 min.; the pleural-abdominal connectives were bathed in "cold" ASW separated from the ganglion by a grease seal. After the pulse period the ganglion and connectives were perfused with aerated chase media (Aplysia culture media containing 10mM cold methionine) for periods of 6, 12, 24, 48, 72 hr, 5d, and 7d at 22° C.

The proteins of fast and slow axonal transport as well as those proteins with long resonant times in the cell body were observed on 1 and 2D-PAGE fluorographs. The complement of fast transported proteins was examined after 6 and 12 hr chase periods by cutting connectives into 2 mm segments and subjecting labelled polypeptides to gel electrophoresis, as well as by collecting fast transported proteins after 24 and 48 hr in segments proximal to ligatures placed on connectives. Proteins of slow axonal transport were obtained by: 1) a "window" analysis for short chase periods, where 500 μm of proximal segments of individual axons (intraganglionic) were avulsed and separated from their cell bodies and 2) assaying proximal segments of connectives at longer chase periods. The 2D analysis indicated distinct profiles of fast and slow transport components with actin, tubulin, NF protein and fodrin being major components of slow transport. Actin and tubulin are among the major cytoskeletal proteins with long resonant times in the cell body. In addition to characterizing proteins which are transported at fast and slow rates, this in vitro system provides a tool for investigating the processing events that may accompany commitment of proteins destined for axonal transport.

237.9 LABELING BY RETROGRADE AXONAL TRANSPORT REVEALS VESICLE HETEROGENEITY IN THE GIANT CEREBRAL NEURON (GCN) OF APLYSIA. Henry B. Kistler Jr.* and James H. Schwartz. Center for Neurobiology and Behavior, College of Physicians and Surgeons of Columbia University, and New York State Psychiatric Institute, New York, N. Y. 10032.

Ultrastructural similarity of many neuronal vesicle types makes it difficult to identify functionally distinct vesicles strictly by morphological criteria. Nevertheless, characterization of functional classes of organelles such as precursors of synaptic vesicles is important. Previous autoradiographic studies with GCN indicated that the transmitter storage granules appear as electron-lucent compound vesicles in the cell body and proximal axon of this serotonergic neuron, and that vesicles with the morphology of compound vesicles are a major component of the membranes traveling along the axon by fast anterograde transport. We now find that morphologically similar vesicles are labeled in the cell body and axon of GCN transporting a wheat germ agglutinin–horseradish peroxidase conjugate (WGA-HRP) in the retrograde direction. We recently described the colchicine-sensitive retrograde axonal transport of WGA-HRP: 18h after the conjugate was injected extracellularly into the neuropil of the buccal ganglion, HRP-reaction product was seen in compound and simple vesicles, multivesicular bodies and cisternal elements of GERL in GCN's cell body (Kistler and Schwartz, Brain Res., in press). In the axon, HRP-positive compound vesicles as well as single vesicles and multivesicular profiles were also present, presumably traveling by retrograde axonal transport. We suggest that the labeled somatic vesicles (and possibly some axonal vesicles too) participate in lysosomal processing because somatic compound vesicles, which are indistinguishable in size from those labeled by WGA-HRP, contain acid phosphatase activity (as do elements of GERL, multivesicular bodies and large end-stage lysosomes). Moreover, compound vesicle are also present in identified cholinergic and histaminergic cells of Aplysia whose transmitter storage organelles differ considerably from those of GCN. These observations suggest that GCN contains at least two classes of compound vesicles. One is the characteristic serotonergic storage and transport granule; the other, a lysosomal organelle that can be labeled by retrogradely transported WGA-HRP and acid phosphatase reaction product, is shared by other identified neurons.

237.10 IDENTIFICATION OF THE PRINCIPLE ORGANELLE CARRIED BY FAST AXONAL TRANSPORT IN THE GIANT CEREBRAL NEURON OF APLYSIA. Leonard J. Cleary and James H. Schwartz. Center for Neurobiology and Behavior, College of Physicians and Surgeons of Columbia Univ. and New York State Psychiatric Institute, New York, N. Y. 10032.

An important problem still to be solved in axonal transport is the form in which membranes are actually moved. Using electron-microscopic autoradiography, we have studied this problem in the giant cerebral neuron (GCN) of Aplysia. In this neuron, 3H-serotonin had previously been localized to only one type of axonal oganelle, an electron lucent compound vesicle. Intrasomatic injection of 3H-fucose has now been used to label axonal membranes. The posterior lip nerve was sectioned 3 h after injection through a region shown to contain the moving front of membrane, and also 2 h later, when the front had passed through. Analysis of the autoradiographs from both time points confirms that compound vesicles are the major source of radioactivity in the axon. Smooth membranous profiles, although significantly labeled, contained much less radioactivity.

Channels of a continuous axoplasmic reticulum have been proposed as the vehicle in which membrane moves by fast transport. It was therefore of interest to see if any of the smooth membranous organelles in GCN's axon were actually cross-sections of a longitudinally oriented reticulum. We saw very few tubular elements in GCN's axon. Analyses of serial longitudinal sections revealed that only a small fraction of these tubules continued into adjacent sections. Although it is possible that continuity may have been lost as an artefact of the fixation, this seems unlikely because continuous tubules of endoplasmic reticulum were plentiful in the cell body and axon hillock of the same neuron. The axoplasmic reticulum projected about a millimeter into the proximal axon, becoming more sparse with distance from the cell body. Because we could not find any organelles that might constitute a continuous axoplasmic reticulum in distal regions of the axon along which transport has been shown to occur, membranes must be moved in vesicles or other discrete organelles. We conclude that the bulk of membranes labeled with 3H-fucose is transported as compound vesicles.

237.11 A THIN SPECIALIZED CORTICAL ZONE LINKING THE AXOPLASMIC SURFACE OF THE AXOLEMMA TO FIBROUS ELEMENTS OF THE NEUROPLASMIC LATTICE AT NODAL REGIONS IN VERTEBRATE MYELINATED AXONS. A. J. Hodge* and W. J. Adelman, Jr.. Lab. of Biophysics, NINCDS, NIH, at the Marine Biological Laboratory, Woods Hole, MA 02543.

Electron stereomicrography of relatively thick (0.1-0.5 μm) sections of Bufo peripheral nerve clearly shows that longitudinal elements of the neuroplasmic lattice (neurotubules and neurofilaments) are linked to the inner surface of the axolemma as well as to one another by thin periodically disposed transverse bridges. This appears to be the case for both unmyelinated and myelinated fibers in toad peripheral nerves. In the latter, however, there appears to be specialization of a thin (15-20 nm) subaxolemmal cortical dense zone limited mostly to the Ranvier nodal regions, but also found in the axonal constrictions commonly associated with Schmitt-Lanterman clefts. The fine structure of this specialized layer is best described as a "fuzzy" or velvety coating on the axoplasmic face of the axolemmal membrane. It appears to consist primarily of short filamentous elements (2-4 nm in diameter) periodically arrayed perpendicular to the axolemmal surface and apparently linking the neuroplasmic lattice elements with this surface. The density of packing of these elements is sufficient to form a zone of exclusion of larger axoplasmic elements such as neurofilaments and neurotubules. The relatively high density of this layer relative to that of interstitial axoplasm is also clearly demonstrable by densitometry. It appears also that elements of the subaxolemmal layer form an ordered array in the paranodal regions of Ranvier nodes and there are indications of a correlation between order found in the cytoplasm of adjacent myelin terminal loops and that in the subaxolemmal layer. The structures described were observed in specimens routinely fixed in an EGTA/Mg^{++}/sucrose/PO_4 buffered glutaraldehyde medium followed by OsO_4 treatment, acetone dehydration and epon embedding. Sections were stained with uranyl acetate and lead citrate and examined in a Philips EM400 electron microscope.

238.1 NEURONAL DISCHARGE IN MONKEY CEREBELLAR CORTEX DURING THE COACTIVATION AND RECIPROCAL INHIBITION OF ANTAGONIST MUSCLES OF THE FOREARM. Allan M. Smith, Robert C. Frysinger, Daniel Bourbonnais* and John F Kalaska (SPON: H.H. Jasper). Centre de recherche en sciences neurologiques, Univ. de Montréal, Montréal, Qué., Canada.

Monkeys were trained to exert and maintain an isometric pinch of the thumb and forefinger on a force transducer in order to elicit voluntary coactivation of forearm muscles. The same monkeys were also trained to flex and extend the open hand and to hold a fixed wrist position against either a predetermined counter torque or against a mechanical stop. When a mechanical stop was used the wrist muscle torque was systematically measured. Analysis of EMG activity in the individual forearm muscles showed that both the prime mover muscles as well as all of the synergist muscles were reciprocally active during isometrically maintained wrist position. A few muscles demonstrated bi-directional activity only during wrist movements. Activity from single cells in the culmen simplex area bordering the primary fissure was recorded. Most units in this area responded to cutaneous and proprioceptive stimulation of the hand and forearm. Neurons displaying climbing fiber discharges were identified as Purkinje cells but the remaining units could not be identified. A majority of Purkinje cells (60%) decreased discharge frequency during the coactivation of antagonists in prehension. In contrast, a majority of unidentified neurons (80%) increased discharge frequency during the same cocontraction. Both Purkinje and unidentified cells discharged reciprocally during isometric maintained flexion and extension. A few neurons (3 Purkinje 5 unidentified) showed similar bi-directional increases or decreases in activity during wrist movement but these units discharged reciprocally during isometric holding. This activity was thought to be related to the muscles contracting during the wrist movements in both directions. Significant linear regressions between discharge frequency and static isometric wrist torque were found for several Purkinje and unidentified cells. These relations indicated that the discharge frequency increased monotonically with wrist force in one direction and decreased in proportion to the wrist force in the opposite direction. Taken together these observations suggest three conclusions. (1) The activity of single neurons in cerebellar cortex is related to the activity of individual muscles. (2) The Purkinje cell discharge reinforces the reciprocal inhibition of antagonist muscles. (3) The decrease in Purkinje cell firing during coactivation may permit maximal stiffening of a particular joint due to decreased reciprocal inhibition. These data support the hypothesis that the cerebellar participates in regulating the antagonist muscles in both posture and movement. This research was supported by the Medical Research Council of Canada.

238.2 RESPONSES OF CEREBELLAR CORTICAL UNITS TO STRETCH OF ANTAGONIST MUSCLES. Daniel Bourbonnais*, Robert C. Frysinger, James P. Lund and Allan M. Smith. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

The activity of cerebellar cortical neurons was recorded in chloralose anesthetized cats (50 mg/Kg, i.v.) during stretches of the triceps surae and tibialis anterior muscles. In about one third of the experiments, all nerves of both hindlimbs except those innervating the stretched muscles, were ligated and cut. In the remaining preparations no denervation was performed. The muscles were alternately and simultaneously stretched by as much as 4 mm, at rates up to 55 mm/s. Simultaneous stretch of both antagonists is unphysiological, but was employed to mimic the symmetrical pattern of muscle afferent activity in cocontraction.

Cerebellar neurons were recorded in the anterior lobe from lobules II to the most rostral part of lobule V. Of the 100 units recorded, 14 Purkinje cells, identified by their complex spikes, and 38 neurons not identified, changed their firing frequency in relation to muscle stretch. These cells were grouped according to their modulation of firing frequency in response to muscle stretch. In one group, the activity of four Purkinje cells and fifteen unidentified cells was modified during stretch of one muscle only. A second group including 10 Purkinje cells and 11 unidentified neurons had the same type of response to stretch of either muscle. Most units in these two groups showed an essentially dynamic response to muscle stretch. The members of a third group that included only unidentified neurons (N = 12), increased discharge during stretch of one muscle, and decreased activity with stretch of the antagonist muscle. The discharge of these cells was often maintained during the static phase of stretch (8/12). Moreover, the firing frequency of 4 neurons was significantly correlated (r = 0.67 to 0.97) with velocity and amplitude of stretch. Although isolated stretch of the antagonist reduced firing frequency, these correlation coefficients remained unaltered during the simultaneous stretch of both muscles.

In conclusion, some neurons (presumed interneurons) receive detailed information about muscle length and the rate of length change. However, Purkinje cells do not appear to respond to static changes in muscle length and have only weak responses to dynamic stretch. This research was supported by the Medical Council of Canada.

238.3 RELATION BETWEEN CORRELATION OF CLIMBING FIBER INPUTS TO NEIGHBORING PURKINJE CELLS AND THE CROSS-CONDITIONING OF THEIR RESPONSES TO MOSSY FIBER INPUTS. J.R. Bloedel, T.J. Ebner, Q.X. Yu*, Depts. of Neurosurgery & Physiology, Univ. of Minnesota, Mpls, 55455.

Previous studies have shown that the climbing fiber input to a Purkinje cell modifies its responsiveness to subsequent mossy fiber inputs evoked by natural peripheral stimuli. The present experiments demonstrate that the activation of a climbing fiber input to one cell by a natural peripheral stimulus can be related to an increased responsiveness of a neighboring cell to mossy fiber inputs, an effect called cross-conditioning. The relationship between this cross-conditioning and the correlation between the climbing fiber inputs in the two cells was also assessed. In lobules IV and V of the anterior lobe of adult unanesthetized, decerebrate cats, the activity of 2 or 3 Purkinje cells .1 to 1 mm apart were recorded simultaneously in the surface layer of a folium. In the 32 sets of neurons the simple and complex spikes were discriminated, and their responses to a brief flexion of the ipsilateral wrist were studied. The trials used to construct the simple spike histograms for each neuron were then divided into two groups, (1) trials in which a climbing fiber input was activated by the peripheral stimulus and (2) trials in which it was not. The histograms constructed from these two groups were used to determine whether the cell's responses to the mossy fiber was greater in those trials in which a climbing fiber input was activated, an effect called self-conditioning. A comparable analysis sorted trials based on the occurrence of a climbing fiber input to the neighboring Purkinje cell. In addition, the correlation between the climbing fiber activity of both cells was assessed from the generalized cross-correlation of the complex spike responses. Cross-conditioning was present in 23 of the 32 sets. Of the 18 sets in which both cross-conditioning and the cross-correlation of complex spike responses were assessed, 13 showed cross-conditioning. Positive correlation in their evoked climbing fiber activity was present in all 13 sets. Of the 25 Purkinje cells demonstrating cross-conditioning, self conditioning was present in 22. Although the climbing fiber input to a given Purkinje cell produced self conditioning in 41 neurons, the same climbing fiber inputs were associated with cross-conditioning in only 22 cells. These data show that there is a strong relationship between cross-conditioning of the responses of two Purkinje cells and the correlation in their climbing fiber activity. The findings support the hypothesis that peripheral stimuli are capable of activating synchronous climbing fiber inputs to the cerebellar cortex which produce an increased responsiveness of Purkinje cells in the same cortical region. This work was supported by #1R01-NS 18338-01 and #R01-NS 09447-10.

238.4 PREFERRED TIMING OF CLIMBING FIBER AFFERENT DISCHARGE EVOKED BY NATURAL PERIPHERAL STIMULI. T.J. Ebner, Q.X. Yu* and J.R. Bloedel. Depts. of Neurosurgery & Physiology, Univ. of MN, Mpls, 55455.

Electrophysiologic studies of the inferior olive have revealed its tendency to discharge rhythmically. However, the role or existence of this periodic behavior in information processing in the cerebellum has not been addressed. The purpose of these studies was to determine if natural peripheral stimuli result in rhythmic or periodic discharge of climbing fiber inputs to the cerebellum. In unanesthetized decerebrate cats Purkinje cell unitary activity was recorded extracellularly, and the complex and simple spike activity discriminated. Brief square-wave displacement of the ipsilateral forepaw was applied with a feedback controlled Ling vibrator. The peristimulus time histograms (PSTH) revealed three or four periodic increases in the complex spike discharge. These increases were usually separated by periods of reduced firing frequency. The presence of a periodicity was confirmed by showing that the autocorrelation of the complex spike histogram possessed similar periodic components. Of 146 Purkinje cells studied 96 responded to the forepaw displacement with an increase in their simple spike discharge. Of these cells, 58 demonstrated periodic components in the PSTH. The periods ranged from 100-280 msec, with a mean of 157 msec. The second peak, the so-called "off-response", was found to be unrelated to the termination of the stimulus in many cells, since changing the duration of the stimuli did not affect the time of occurrence of the second peak. Intriguingly, in pairs of neighboring Purkinje cells recorded simultaneously both neurons exhibited the same period although usually slightly out of phase. In responses with several peaks in the PSTH, an examination of single sweeps suggested that three or four complex spikes were not evoked by each stimulus. This was confirmed by sorting the trials from which the complex spike PSTH was generated based on the presence or absence of a complex spike within specific time limits. In most cells the histograms constructed from trials with complex spikes within the window showed a marked reduction in complex spikes for a considerable period outside the window. For example, if the time window was placed to select out the trials in which a complex spike occurred in a specified peak of the complex spike PSTH, no or only a few spikes occurred in the same trials at the time of the other peaks in the PSTH. This study demonstrates that the periodicity to natural peripheral stimuli observed in the complex spike histogram is not the result of sequential activation of climbing fiber inputs to a single stimulus but instead results from climbing fiber activity evoked at preferred intervals, which are hypothesized to reflect the inherent rhythmicity of the inferior olive. Supported by NIH Grants: 2R01-NS 09447 and 1R01-NS 18338.

238.5 A STUDY OF SENSORIMOTOR PROPERTIES OF DENTATE NEURONS DURING CONDITIONED ARM MOVEMENTS IN THE MONKEY. C.E. Chapman, G. Spidalieri* and Y. Lamarre. Centre de recherche en sciences neurologiques, Fac. de méd., Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

The results of electrophysiological and lesion studies indicate that the lateral cerebellar system is involved in the initiation of rapid voluntary movements, especially those triggered by teleceptive stimuli. The purpose of this study was to investigate the role of the dentate nucleus in the initiation of rapid arm movements triggered by different sensory cues.

Two monkeys were trained to perform rapid flexion and extension movements of the elbow in response to 3 different, randomly presented sensory cues: small perturbations of the elbow, light signals or pure tones. Unitary discharge was recorded from 317 cells in the ipsilateral dentate nucleus. Task-related discharge was recorded throughout the rostral-caudal extent of the nucleus. In 1 monkey, the discharge of 72% of the cells (152/209) was modulated during the task and in 77% (118), the earliest change preceded the onset of movement (mean latency 122 ms [n = 237] for the 2 monkeys). In most cells, the pattern of discharge before movement onset was independent of direction. A small number (20%) showed greater modulation in one direction. Reciprocal changes were observed in 4 neurons, but only after movement onset.

For units in which the initial change preceded movement, the discharge pattern was classified as follows. In 33% of the cells, the earliest change in activity was clearly related to the onset of movement and was correlated with parameters of movement. In 66% the initial change was linked to one or more of the sensory cues (mean latencies: perturbation and light - 80 to 90 ms; sound - 60 to 80 ms). These "sensory"-related cells were almost entirely restricted to the caudal one-half of the nucleus. In one-third, a similar response was observed with all 3 cues. The response often ended at the onset of movement and was correlated with movement parameters. Two-thirds of the "sensory" related neurons responded specifically to only 1 or 2 of the cues, most often light and/or sound. The onset and duration of the response were, however, strongly correlated with reaction time. The "sensory" response was context-dependent - when movement was extinguished by withholding the reward, the response was abolished. In 25% of the cells in each category, there was a strong correlation between activity during movement and movement parameters. This was not thought to reflect peripheral feedback since no cells showed any clear peripheral receptive field. The properties of the early "sensory" response suggest that this represents a gating mechanism for the initiation of movement. This is supported by results from lesion studies which have shown that dentate lesions delay reaction time movements triggered by teleceptive, but not somesthetic stimuli. (Supported by the Medical Research Council of Canada).

238.6 EFFECTS OF SUSPENDING CLIMBING FIBER ACTIVITY ON THE DISCHARGE PATTERNS OF FLOCCULAR PURKINJE CELLS. C. S. Leonard* and J. I. Simpson (SPON: R. I. Frank). Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Many Purkinje cells (P-cells) of the rabbit flocculus exhibit modulation of both their simple spike (SS) and climbing fiber (CF) activity in response to rotation of the visual world. Typically, movement in a direction that increases the CF firing rate decreases the SS firing rate and vice versa. In lightly anesthetized, paralyzed rabbits we investigated the effects of suspending the CF input on both the spontaneous SS activity and the SS modulation induced by visual stimuli. Prior to recording extracellularly from single floccular P-cells, a pipette filled with 2% lidocaine HCl was placed in the contralateral caudal dorsal cap of the inferior olive, which is the source of floccular CF's responsive to visual world rotation about the vertical axis. The P-cells studied were those whose CF's had receptive field properties that corresponded to those of the inferior olive neurons near the tip of the lidocaine pipette. This arrangement readily allowed P-cell CF activity to be blocked by pressure injection of 5-40 nl of lidocaine. CF activity stopped within a few seconds after lidocaine injection and was absent for about two minutes; longer periods of silencing were achieved by repeated injections of lidocaine. Both the SS and CF activity of P-cells were represented by histograms (100 msec binwidth) compiled in the absence of visual stimulation and during periodically reversing, constant speed stimulation (10 sec period, 15 repetitions). Within four minutes after blockage of CF activity, the spontaneous SS rate (averaged over 150 sec) increased by 7-18 spikes/sec in 8 out of 11 P-cells. This increase, as a percent of pre-injection spontaneous SS rate (25-90 spikes/sec), ranged from 8 to 34%. During the period of CF suspension the visually induced modulation of SS activity was still present. In fact, upon comparison of the histogram profiles of the SS modulation obtained before and during CF blockage, we were struck more by their similarity than by their dissimilarity. Although this particular analysis has not revealed a high degree of interaction between SS and CF activity, it is possible that cessation of CF activity engenders as yet undetected changes in the finer-grained statistics of SS activity. Supported by USPHS grant NS13742 from NINCDS.

238.7 SIMULTANEOUS SAMPLING AND ANALYSIS OF THE ACTIVITY OF MULTIPLE, CLOSELY ADJACENT CEREBELLAR PURKINJE CELLS. J. Bower and R. Llinas. Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., New York 10016.

Present morphological and physiological data concerning the structural organization of the cerebellar cortex suggests that its neural networks process information in an intrinsically parallel fashion. That is, the output of the cerebellar cortex is dependent on the simultaneous and interrelated activity of large arrays of cerebellar Purkinje cells (PC's). While this principle has been incorporated into theories concerning functional organization of the cerebellar cortex (Pellionisz & Llinas, Neuroscience, 4:323, 1979), insufficient physiological data are available on the real-time relationships of activities in multiple functionally related PC's to confirm or negate such views. This is mainly due to the lack of adequate methods for the simultaneous sampling and analysis of the electrical activity of multiple, functionally related PC's.

We report an experimental approach allowing simultaneous extracellular recording from multiple PC's within highly restricted areas of the cerebellar cortex of albino rats. Our methods permit 16 or more microelectrodes to be positioned individually to record from PC's within a square mm of cerebellar cortex. The electrodes, which are "psuedo-floating", can record extracellular potentials from single PC's in a very stable manner for hours. For signal processing, we have designed and constructed sets of 16-channel head stages compact enough (2.5 x 3.5 x 5.5 cm) to locate near the preparation, as well as rack-mounted 16-channel processing amplifiers which convert each recorded PC spike train into a series of computer compatible TTL pulses. To display simultaneously the spontaneous or evoked spike activity of all sampled PC's, the TTL output from each channel is used to drive one of 16 LEDs and/or pizoelectric buzzers. Each LED and buzzer combination is independently positioned on a display board to reproduce the actual spatial distribution within the PC layer of the sampled PC's. Further analysis of the data is currently done in one of two ways: 1) the TTL pulse output of each of the 16 channels is summed and/or integrated in real time in a manner that simulates the integrations performed by the deep cerebellar nuclear cells; 2) the processing amplifier is interfaced directly to a VAX computer for comparisons to predicted results by theoretical models (Pellionisz & Llinas, Neuroscience, 4:323, 1979), and for conventional off-line analysis and data storage. Supported by USPHS grants NS06958 and NS13742.

238.8 MICROSTIMULATION OF THE CEREBELLAR VERMIS AND SACCADIC EYE MOVEMENTS. James G. Mc Elligott and Edward L. Keller, Department of Pharmacology, Temple Univ. Schl. of Med., Phila., PA 19140 and Smith Kettlewell Inst. of Vis. Sci., San Francisco, CA 94115.

Previous work on electrical stimulation of the cerebellar vermis indicated that saccadic eye movements are topographically organized (Ron & Robinson, 1973). However, single cell recording experiments in both the awake cat (Waterhouse & Mc Elligott, 1980) and monkey (Mc Elligott & Keller, 1981) did not reveal any such organization. In order to reconcile these differences, a microstimulation study was undertaken in the two monkeys that had been used in the previous single unit recording study. Thus, results employing the two techniques could be directly compared in the same animals. Water deprived monkeys were trained to fixate for 1 second on a visual target located on one of the primary axes. During this fixation period, electrical stimulation (max. current = 300 μA) was delivered via a microelectrode. Microstimulation in the area of the vermis revealed a topographical organization of the evoked saccades as previously reported. However, along each electrode track there was a point of minimal threshold (20-50 μA) that was always located in the white matter. Stimulation of the superficial cortical cell body areas involved with saccadic eye movements at maximum current often did not evoke any saccades. Thus, there was never any overlap between the locations where saccadic related cells were recorded and these points of minimal threshold. These results indicate that electrical stimulation in the cerebellum operates by direct stimulation of the input and/or output elements. Stimulation of the cortical cell body areas for currents 300 μA and greater appear to be evoked by current spread to these points of minimal threshold that lie at the confluence of the input and output fibers for the folia.

This study also revealed that the presence or absence of a saccade as well as its magnitude is dependent on eye position at the time of electrical stimulation. This position dependency property of the cerebellar vermal evoked saccades has also been revealed by previous single unit and lesion studies in this area. (Supported by N.S.F. Grant #79-14107).

238.9 EYE MOVEMENTS EVOKED BY MICROSTIMULATION IN THE FLOCCULUS OF THE MACAQUE. D. B. Belknap*, H. Noda and M. Ohno*. School of Optometry, Indiana Univ., Bloomington, IN 47405.

Observations of Purkinje cell activity during oculomotor behavior have indicated a role for the cerebellar flocculus in the control of eye movements. In order to corroborate these findings, we investigated the effects of modification of Purkinje cell discharges using microstimulation. The pattern of the stimulus pulse-train was based on the floccular output signals recorded from nearby Purkinje cells in pig-tailed macaques which moved their eyes with a visual target. The molecular layer was identified during recording by the presence of climbing fiber responses from Purkinje cell dendrites, the granular layer was characterized by abundant mossy fiber discharges during saccades, and the Purkinje cell layer was identified by a marked increase in the background activity with multiple cellular spikes. When eye movement-related Purkinje cell discharges were encountered, the threshold (current necessary to evoke eye movements) was measured systematically at 50 μ intervals, using a 0.5 sec, 200 Hz train of 0.5 msec biphasic pulses. The current necessary to elicit eye movement was occasionally as low as 3 μA. Low threshold sites such as these were found either in the molecular or granular layer, and extended for 100 μ along the track, with rapidly rising thresholds outside the site. Even without moving the electrode, an increased stimulus current could alter the direction of the eye movements. In response to 500 msec, 10 μA pulse-trains, the elicited eye movements were typically slow eye movements (SEMs) of 5^{o} or less. Velocity was correlated with stimulus frequency and ranged from 1 to 20^{o}/sec. The SEMs began at a latency of 10-20 msec and continued in the opposite direction after a similar delay following the end of stimulation. We observed SEMs in all directions, and the direction of the SEM could vary from site to site even within a single track. When a long, sinusoidally frequency-modulated pulse-train was applied, the eyes moved at a velocity which varied sinusoidally at the fundamental frequency of the pulse-train (0.3 to 2.0 Hz). Peak eye velocity appeared in synchrony with the highest stimulus frequency at some sites, and with a phase shift of 90^{o} at some others. Saccadic eye movements were also elicited by microstimulation. However, stimulus thresholds were higher for saccades than for SEMs, and the direction of the saccades was roughly opposite to that of the SEMs. Saccades appeared after the cessation of brief pulse-trains or after a considerable delay following the stimulus onset, and recurred repeatedly, if the stimulus was continued. The inter-saccadic interval was less at higher stimulus frequencies. (Supported by NIH Grant EY04063)

238.10 ADAPTATION TO PRISM DISPLACEMENT OF VISION: EVIDENCE FOR THE ROLE OF CEREBELLUM IN MOTOR LEARNING. M. Hallett, M. Weiner* and H. H. Funkenstein*. Section of Neurology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

The visual-motor adaptation to lateral displacement of vision by prism glasses was studied in seven patients with cerebellar dysfunction, 10 with Parkinson's Disease, 14 with right cerebral hemisphere deficits, 15 with left cerebral hemisphere deficits, 11 with Alzheimer's Disease, and 3 with Korsakoff's Syndrome and compared to 20 normal subjects. The process of adaptation was analyzed in two phases: the return to normal pointing with prism glasses in place, called the error reduction portion, and the mispointing in the opposite direction after the glasses were removed, called the negative aftereffect portion. The adaptive process is viewed as a form of motor learning.

Error reduction was impaired in the cerebellar, Parkinsonian, right cerebral hemisphere, and Alzheimer groups. This impairment may not reflect a derangement of adaptation since a failure of cognitive correction may also operate in this portion. Negative aftereffect was reduced only for the cerebellar patients. We contend that the negative aftereffect portion better reflects the process of adaptation itself, being less influenced by cognitive correction. The poor performance of the cerebellar patients is thought to be evidence for the involvement of the cerebellum in the process of motor learning.

238.11 DURATION OF INITIAL AGONIST EMG ACTIVITY IN RAPID MOVEMENTS IN PATIENTS WITH CEREBELLAR DEFICITS. J. Matheson*, A. Berardelli*, R. Weinhaus* and M. Hallett (SPON: H. R. Tyler). Section of Neurology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

The EMG correlate of a rapid limb movement begins with a discrete burst of activity in the agonist muscle. The duration of this burst does not vary significantly with movements of different distance for normal subjects. Patients with cerebellar deficits have been shown to have prolonged burst lengths when tested at a single distance (Hallett et al., J Neurol Neurosurg Psychiat 38: 1165,1975). The purpose of the present study was to investigate whether there was any significant change in burst duration with differing distance in these patients.

Attempted rapid movements of the elbow of 10, 20 and 30^{o} were made while recording EMG from biceps (and triceps) in seven patients. Similar experiments were done with rapid movements of the top joint of the thumb of 5, 10 and 20^{o} while recording from flexor pollicis longus in four patients. Durations of EMG bursts were determined by visual inspection and related for each movement to the actual distance moved (as opposed to the distance attempted). Increases in burst duration with increasing distance were found for all patients at both joints and this was statistically significant in all but one patient. Increases varied from about 20-100%.

Since cerebellar patients make hypermetric movements and EMG burst duration increases with movements of greater distance, presumably the movements would not be hypermetric if the EMG burst duration were shorter. Hence it appears that the abnormality of increased burst duration could be etiologic in cerebellar hypermetria.

239.1 PARTIAL PURIFICATION OF THE RECEPTOR FOR GABA. C.J.BARNSTABLE and R.HOFSTEIN. Dept. of Neurobiology ,Harvard Medical School, Boston, MA 02115.

Purification of membrane receptors is an inevitable step towards the understanding of synaptic transduction at the molecular level. GABA is a major inhibitory neurotransmitter and its receptor is fairly abundant in many brain regions. However, the molecular mechanisms underlying the inhibitory responses of GABA are as yet unclear. Better understanding of gabaergic mechanisms can be achieved, in part, by isolation and purification of the receptor.

Membrane fractions (P2M) were isolated from rat forebrains, washed extensively and treated with 0.05% Triton X-100 to drop endogenous GABA levels from 1000pmole/mg to below 20pmole/mg. GABA binding was detected in plain P2M and Triton-treated membranes by binding assays using ^{3}H-Muscimol. The binding was saturable and displacable by bicucculine (IC50= $5x10^{-7}$) and GABA (IC50= $5x10^{-8}$). Scatchard analysis revealed the presence of two binding sites (Bmax= 2.4 pmole/mg with KD= 133nM; Bmax=0.5 pmole/mg with KD= 1.8nM) in the plain P2M fraction and a single site(Bmax= 2.1 pmole/mg with KD= 11nM) in the Triton treated membranes.

P2M (3mg/ml) fractions initially treated with 0.05% Triton, were solubilised with various detergents. Both 2% deoxycholate and 2% sodium cholate solubilised as much as 85-99% of the membrane proteins. 2% and 1% Triton X-100 solubilised 65% and 50% of the proteins, respectively. 1% Digitonin, 0.5% lysolecithin and 1% Nonidet P-40 each solubilised only 40-50% of the proteins. All soluble fractions were then diluted to reduce the detergent concentration. Binding of ^{3}H-Muscimol was detectable in the soluble fraction following solubilization with lysolecithin, Triton, cholate and deoxycholate treatment. The binding was saturable and Scatchard analysis revealed a single binding site (Bmax= 800-1200fmole/mg, KD= 24nM). Most binding sites remained in the membrane pellets except for deoxycholate and cholate. Due to the efficiency of solubilization with sodium cholate we have used it for further purification studies. Three fold purification was achieved by gel filtration on Bio-gel A1.5 (150fmole/mg protein as compared to 50 fmole/mg in the original soluble fraction, using 1.0nM ^{3}H-Muscimol). The apparent molecular weight was 600,000-850,000 daltons relative to globular marker proteins. Fifteen fold purification was achieved by affinity chromatography on lentil(Lens culinaris) lectin conjugated to Sepharose 4B. These intial results will lead to the purification of the GABA receptor and will allow a better understanding of the gabaergic system at the molecular level. (Supported by Chaim Weizmann fellowship to RH and grants NS17309 and EY03735).

239.2 RECONSTITUTION OF STRIATAL DOPAMINE RECEPTORS INTO PLANAR LIPID BIMOLECULAR MEMBRANES. R.B. Murphy and V. Vodyanoy, Department of Chemistry and Laboratory of Radiation and Solid State Physics, New York University, 4 Washington Place, New York, N.Y. 10003.

The reconstitution of receptor-linked ionic channels into planar lipid bimolecular membranes has recently become a useful approach toward the understanding of the physiological function of membrane-associated receptors. We have applied this methodology to the functional incorporation of dopamine receptors from rat striatal homogenates into essentially solvent-free lipid bimolecular membranes of large surface area. Briefly, striata were homogenized in 10 mM MOPS-50 mM sucrose, pH 7.40 (K^+-counterion) and after a low speed (900 xg) spin the homogenate was sedimented and resuspended four times at 100,000 xg (4°C) in the same buffer. The resultant pellet was sonicated for 2 minutes at 0°C in order to form vesicles capable of fusion with a planar lipid bimolecular membrane. After incorporation at 25°C into an egg phosphatidylcholine membrane (essentially solvent free; bathing electrolyte 20 mM KCl, 20 mM NaCl, 2 mM $CaCl_2$, pH 7.40, ~ 1 mm^2, area), the system was either treated with (+)-butaclamol (100 nM), (-)-butaclamol (100 nM), or was untreated. To these systems was added the dopamine agonists apomorphine and ADTN over the concentration range 1 - 500 nM. In a series of 12 independent experiments, the relative DC conductance of the apomorphine treated membrane increased in a dose dependent manner, if the system was previously untreated or treated with (-)-butaclamol. The presence of (+)-butaclamol (100 nM) completely eliminated the response within the limits of experimental precision. Similar results were observed with ADTN. Significant perturbation of membrane capacitance was not observed within the duration of individual experiments. Scatchard analysis of the apomorphine data indicated a K_d in the range of 1-1.5 nM, which is in agreement with values observed using (^{3}H)-apomorphine binding as measured with radioreceptor assays.

Studies using bathing media with various supporting ions suggest a specific ionic basis for these phenomena.

Supported by NSF BNS-8118761, NBS NB81 NADA007, and NIMH.

239.3 CHARACTERISTICS OF GLYCINE-ACTIVATED CHANNELS IN LAMPREY BRAINSTEM NEURONS. A. R. Martin and M. R. Gold. Dept. of Physiol. Univ. of Colorado Sch. of Med., Denver, CO 80262.

Glycine has been shown previously to increase Cl^- conductance in Müller cells in the brainstem of the lamprey and to mimic the natural inhibitory neurotransmitter (Matthews, G. & Wickelgren, W. O., J.Physiol.,Lond. 293: 393, 1979). We have used techniques of noise analysis to deduce the mean conductance (γ) and mean open time (τ) of individual glycine-activated channels. Brains were removed from anesthetized lampreys and superfused with a cold (3° to 10°C) saline solution containing tetrodotoxin and 4-aminopyridine. Individual cells were impaled with two micropipettes for voltage clamping. The electrodes were filled with 3 M K^+-acetate and had resistances in the range of 20 to 30 MΩ. Glycine was applied to the cells near the base of the dendrites from a third pipette filled with a 0.2 to 1.0 M solution of the drug. Glycine application produced membrane currents in the voltage-clamped cells, accompanied by marked increases in current variance ("noise") over baseline levels. The spectral density of this increase in variance was used to calculate values of γ and τ for the underlying conductance channels. These were found to be 73 ± 14 pS and 34 ± 9 msec, respectively (mean ± S.D., N = 15). The mean reversal potential for the drug effect was -65 ± 7 mV, and neither γ nor τ was strongly dependent on membrane potential. When Cl^--filled electrodes were used for clamping, intracellular Cl^- concentrations were increased and reversal potentials shifted to the range of -30 to -55 mV. This increase in intracellular Cl^- resulted in an unexpected decrease in channel conductance to 21 ± 9 pS (N = 7). When intracellular Cl^- was increased by raising extracellular K^+ (rather than by using Cl^--filled electrodes) there was a similar reduction in γ to 30 ± 9 pS at a mean reversal potential of -47 mV. Decreasing external Cl^- in the high K^+ solution by partial replacement with isethionate decreased internal Cl^- and increased γ to 52 ± 6 pS (N = 6) with no significant change in mean reversal potential (-49 mV). In contrast to these effects on γ, there was no significant change in τ with changes in Cl^- concentrations. Thus, it appears that in these channels increased intracellular Cl^- reduces channel conductance without affecting channel kinetics.

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239.4 ANALYSIS OF INHIBITORY POSTSYNAPTIC CURRENTS IN THE BRAINSTEM OF THE LAMPREY. M. R. Gold and A. R. Martin. Dept. of Physiol. Univ. of Colorado Sch. of Med., Denver, CO 80262.

Spontaneous inhibitory postsynaptic currents (i.p.s.c.'s) were studied in Müller cells in the lamprey brainstem to compare the channels activated by the natural transmitter with those activated by glycine. Brains were dissected from anesthetized lampreys and perfused with saline at 3° - 10°C. Müller cells were impaled with two K^+-acetate-filled micropipettes for voltage clamping. In normal saline spontaneous i.p.s.c.'s were often observed. These currents were due presumably to presynaptic action potential activity as they were abolished by tetrodotoxin (TTX). Analysis of 44 i.p.s.c.'s from 4 cells revealed a mean peak conductance of 105 nS and reversal potentials (-68 mV), time constants of decay (32 msec) and sensitivities to strychnine (20 μM) that were indistinguishable from comparable measurements of glycine-activated channels (-65 mV, 34 msec and 20 μM, respectively). The conductance measurments suggested that these currents were due to the synchronous activation of about 1500 such channels. When the preparation was bathed in saline containing elevated levels of K^+ (11 mM), spontaneous miniature i.p.s.c.'s were observed which were TTX-resistant. The i.p.s.c.'s showed normal distributions of amplitudes and time constants of decay, and these measures were uncorrelated. Analysis of 76 events from 3 cells revealed a mean peak conductance of 46 nS and time constant of decay of 30 msec. The reduction in peak conductance (41% of normal) was comparable to that seen for glycine-activated channels in elevated K^+ saline (44%), as was the change in reversal potential (to -46 mV vs. -47 mV). In elevated K^+, low Cl^- (25% of normal) solutions, miniature i.p.s.c.'s had a mean peak conductance of 69 nS and decay time constant of 30 msec. Again, there was good correspondence between the i.p.s.c.'s (N = 30) and the glycine-activated channels with respect to the reduced conductance (70% vs. 65%) and the reversal potential (-48 mV vs. -49 mV). In summary, both glycine responses and i.p.s.c.'s were blocked by strychnine and the mean relaxation time for the glycine-activated channels was the same as the time constant of decay of the i.p.s.c.'s. The effects of varying Cl^- levels on conductance were indistinguishable for the two responses. These observations provide further evidence for the identification of glycine as the inhibitory transmitter in these cells. Furthermore, the TTX-sensitive i.p.s.c.'s and the K^+-evoked miniature i.p.s.c.'s appear to be equivalent, suggesting that the presynaptic action potentials release at most a single quantum of transmitter. This indicates that the mean quantal content is less than unity at these synapses.

(Supported by N.I.H. Grants Nos. NS-09660 and NS-06283)

239.5 PHARMACOLOGICAL MODULATION OF GABA RESPONSES IN CULTURED MOUSE SPINAL NEURONS. D. Owen*, R. Study, E. Gratz*, and J.L. Barker. Laboratory of Neurophysiology and Epilepsy Section, NINCDS, NIH, Bethesda, MD 20205

Augmentation of GABA-induced increases in Cl^- conductance has been proposed as a mechanism of action of several classes of anticonvulsant and CNS depressant drugs. We have found, using cultured mouse spinal neurons, that most of the commonly used anticonvulsant drugs do not affect GABA responses at therapeutically relevant concentrations, but several drugs which have been reported to interact with benzodiazepine (BZ) binding can alter GABA responses. Phenytoin (200 μM), phenobarbital (200 μM), valproate (1 mM), and ethosuximide (1 mM) did not affect GABA responses, while clonazepam (1 μM), augmented responses in the same way as other clinically effective BZs which share its sedative-hypnotic properties. The BZ response was blocked by the BZ receptor antagonists RO 15-1788 and RO 14-7437 (50 μM). The triazolopyridazine, CL 218,872 (10 μM) which has been reported to bind to BZ receptors with a preference for the BZ1 subclass, did not augment GABA responses. RO 5-4864 (10 μM), which has a high affinity for non-neuronal BZ sites, was a potent inhibitor of GABA responses. 3-hydroxymethyl beta-carboline (50 μM) which has been reported to antagonize certain anticonvulsant effects of diazepam, weakly inhibited GABA responses but did not block the ability of BZs to enhance GABA action. The 3-t-butyl ester of beta-carboline (10-50 μM), which itself antagonizes pentylene tetrazol-induced convulsions (much like BZs), augmented GABA responses. The 3-methyl ester of beta carboline (10-50 μM), which is a convulsant, antagonized GABA responses. Etazolate (SQ-20009), a pyrazolopyridazine which enhances BZ binding, augments GABA (1-100 μM), and at higher concentrations (greater than 100 μM), produces a direct increase in membrane Cl^- conductance, both effects resembling those of pentobarbital, a drug which also enhances BZ binding. In conclusion, we have found that the ability to augment the postsynaptic increase in Cl^- conductance produced by GABA is not a property common to all drugs with anticonvulsant activity. However, several drugs which interact with BZ receptors appear to demonstrate a relationship between their ability to modulate GABA responsivness of cultured spinal neurons, and pro - or anti-convulsant activity in vivo.

239.6 IDENTITY OF THE LATE HYPERPOLARIZING POTENTIAL RECORDED INTRACELLULARLY IN RAT HIPPOCAMPAL PYRAMIDAL CELLS IN VITRO. B. E. Alger. Univ. of Maryland Sch. Med., Baltimore, MD 21201

Orthodromic activation of CA1 pyramidal cells produces an EPSP followed by a complex hyperpolarizing response consisting of an early GABA-mediated IPSP and a late hyperpolarizing potential (LHP). Nicoll and Alger (Science, 212:957, 1981) suggested that the LHP is a Ca-dependent K-potential. The LHP is neither GABA-mediated nor Cl^--dependent, but its Ca-dependence is not firmly established and the means by which it is initiated is not understood. For example, the proposal has been made that the LHP represents the activation of a Na/K pump. I have found that while ouabain (0.5-1.0 μM) does block the LHP, it also blocks GABA-mediated IPSPs an indirect effect due in part to a shift in E_{IPSP}. Thus the action of ouabain is inconclusive.

The present evidence indicates the LHP is Ca-dependent and results from synaptic activation of pyramidal cells: 1) Raising bath Ca concentrations over the range 2.5-10 mM causes a pronounced increase in LHP amplitude. 2) In the presence of picrotoxin IPSPs are blocked and in many cells subthreshold stimulation produces an EPSP-LHP sequence. When recorded with 2M CsCl filled electrodes the cells can be depolarized to 0- +20 mV. At these levels all regenerative activity is usually blocked and the EPSP is reversed, however a Ca-dependent LHP can still be evoked. 3) In five cells recorded in picrotoxin with electrodes containing CsCl plus 0.2M EGTA very long bursts (>5sec) were evoked at resting potentials. When these cells were depolarized to about +10 mV the LHP was either very reduced or absent. 4) Diffusion of Cs into cells rapidly blocks "voltage-dependent" afterhyperpolarizations (AHPs). Although LHPs are eventually depressed by intracellular Cs they are more resistant than AHPs. Interestingly, extracellular perfusion with Cs (3-5 mM) readily blocks LHPs, but not EPSPs. Since Cs is thought to block K channels these data support the conclusion that the LHP is K-dependent. Whether differential sensitivity of the LHP to extra- versus intracellular Cs is a property of the K channels, or just means that extracellular Cs has easier access to the channels, is not clear. 5) Finally, spontaneous EPSP-LHP sequences recurring at 0.1 Hz can be recorded in CA1 cells bathed in GABA antagonists, i.e., the particular cell recorded may not burst. "Spontaneous" activity in CA1 is actually driven by CA2/CA3 (Schwartzkroin and Prince, Brain Res., 147:117, 1978) so it appears that the LHP is closely coupled to activity of the Schaffer collateral system.

These results are consistent with the hypothesis that the LHP is a synaptically activated Ca-dependent K-potential. In addition the fact that the LHPs produced under the various conditions described above all appear to be identical means that the LHP can now be studied free from IPSP or burst contamination. NIH Grant NS17539.

239.7 HOW DO MUSCARINIC IPSPS INFLUENCE INTEGRATIVE PROPERTIES OF SYMPATHETIC NEURONS? J.P. Horn and J. Dodd* Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

In C neurons of the 9th and 10th paravertebral chain ganglia of bullfrogs, a muscarinic ipsp is produced by a 2 to 4 nS increase in membrane K^+ conductance(Horn & Dodd, Nature, 292,625, 1981). The present experiments were designed to determine how effectively this synaptic potential influences the firing properties of C cells. Conventional intracellular recording methods were used.

When single action potentials were elicited either by a suprathreshold nicotinic epsp or by an antidromic stimulus, they were unaffected by ipsps. However, near the normal resting potential of -50mV, C cells will fire repetitively in response to pulses of depolarizing current applied through the recording microelectrode. The ipsp blocks this firing. In addition to the nicotinic epsp and the muscarinic ipsp, a third synaptic potential, that is insensitive to both d-tubocurarine and atropine, can be elicited in C cells. This response, a slow epsp, appears to be mediated by a peptide resembling LHRH(Jan, Jan & Kuffler,PNAS,76,1501,(1979), PNAS,77,5008,(1980)). During the slow epsp the tendency for C cells to fire repetitive action potentials is enhanced. Repetitive firing induced by the slow epsp also was inhibited by the ipsp. Interaction between the muscarinic ipsp and the peptidergic epsp thus resulted in phasic bursting behavior that was of synaptic origin.

The powerful influence of the ipsp upon repetitive firing might have been predicted from the observation that applied currents as small as 25 pA can stop repetitive firing. At a resting potential of -50 mV, there would be 50 mV of driving force on K^+ ions and a muscarinic conductance increase of 2 nS would generate 100 pA of outward current.

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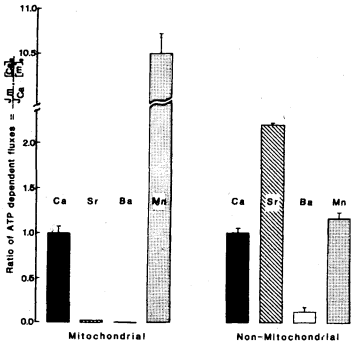
239.8 ATP-DEPENDENT UPTAKE OF Ca, Sr, Ba AND Mn BY INTRACELLULAR ORGANELLES IN SYNAPTOSOMES. H. Rasgado-Flores., D. A. Nachshen and M. P. Blaustein.Dept.Physiol. U.Md, Sch.Med. Baltimore,Md. 21201.

We compared the ATP-dependent buffering of Ca, Sr, Ba and Mn by the mitochondrial and non-mitochondrial (presumably the smooth endoplasmic reticulum, S.E.R.) organelles, in pinched-off nerve terminals (synaptosomes). Rat fore-brain synaptosomes were treated with saponin in order to expose the intra-synaptosomal organelles to the experimental solutions. Radiotracer uptake was determined with a filtration technique, after incubating the saponin-treated synaptosomes (1 min) in media containing (mM): KCl,150; Hepes,20; $MgCl_2$,1.4; KH_2PO_4,2 (for Sr experiments); with or without mitochondrial blockers (oligomycin,0.07 μg/ml; Na-azide,0.2 mM; DNP,0.2 mM); and either ^{85}Sr,0.02; ^{133}Ba,0.02; or ^{45}Ca,0.02, at pH 7.0. The ^{54}Mn (0.54 mM) solutions were of a similar composition except that 0.5 mM ATP was used and $MgCl_2$ was omitted; control ^{45}Ca (0.056 mM) solutions contained 0.5 mM ATP and 5 mM $MgCl_2$. These values were calculated to maintain the free Ca and Mn concentrations at comparable levels (0.05 mM). ATP-dependent uptake was estimated as the uptake in the presence of ATP minus the uptake in its absence. The mitochondrial component of uptake was estimated by substracting the ATP-dependent uptake in the presence of mitochondrial blockers from the ATP-dependent uptake in the absence of the blockers. The figure below shows the ATP-dependent uptake of Ca (J_{Ca}) relative to that of each test divalent cation (J_m), normalized for the external divalent ion concentrations. The sequence for mitochondrial uptake of divalent cations was Mn>>Ca>Sr>Ba, whereas the sequence for non-mitochondrial (S.E.R) uptake was Sr>Mn=Ca>Ba. The differences in intracellular buffering as well as the differences in the rate of extrusion (Sánchez-Armáss et al, this volume) may help to explain the differing effects of these divalent cations on the time course of evoked neurotransmitter release.



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239.9 EFFLUX OF Ca,Sr,Ba AND Mn FROM NERVE TERMINALS. S.Sánchez-Armáss*, D.A.Nachshen and M.P.Blaustein. Dept. of Physiology, Univ. of Maryland. Med.Sch. Baltimore, MD 21201 and Unidad de Investigaciones Biomédicas. Centro Médico Nacional. AP 73-032. México D.F.

Ca efflux plays an important role in regulating the intracellular Ca concentration in nerve terminals. Since several divalent cations substitute for Ca in supporting evoked transmitter release, we studied the efflux of Sr, Ba and Mn in synaptosomes (pinched-off presynaptic nerve terminals). Synaptosomes were incubated for 10 sec. in depolarizing K-rich solutions that stimulated divalent cation uptake. To obtain similar loads for all the ions, the loading solutions contained either (in μM) Ca,20; Sr,60; Ba, 150; or Mn,150; labeled with ^{45}Ca, ^{85}Sr, ^{133}Ba or ^{54}Mn, respectively. The uptake was terminated by diluting the suspension 16 fold with ice-cold Na- and Ca-free solution. Since low temperatures inhibited radiotracer efflux, the initial loads were determined by immediately filtering the diluted samples. The filters were washed twice with cold Ca-free 145 mM Na solution with 2 mM $LaCl_3$. The protocol for measuring efflux was similar, except that warm (30 °C) diluting solutions containing different concentrations of Na, and either Ca or EGTA were used. After 2-30 sec., the samples were filtered as above; the decrease in retained radioactivity compared to the initial load was taken as a measure of efflux.

In the absence of Na ($N-CH_3$-Glucamine or Choline substitution), efflux of Ca, Sr, Ba or Mn in 20 sec. at 30 °C amounted to 20-30 % of the initial load; efflux was abolished at 4 °C.

Sr, like Ca, was extruded at a higher rate (50 % loss in 20 sec.), when the diluting solution contained Na but not Ca. The initial rate of Na-dependent Sr efflux was very similar to the initial rate of Ca efflux (10-15 %/sec.). There was, however, negligible Na-dependent efflux of either Mn or Ba.

The addition of 1 mM Ca to a Na-free solution also caused about 50% loss in 20 sec. of the initial Ca or Sr load, but only about 20 % loss of Ba or Mn.

These results indicate that Sr, but not Ba or Mn utilizes the Na/Ca exchange mechanism located in the nerve terminal plasma membrane. All three ions however, can be exchanged for Ca.

The differences in extrusion of the four cations, as well as the differences in intracellular buffering (Rasgado Flores et al., this volume) may help to explain the differences in the time course of their effects on transmitter release.

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239.10 CASTELLIC ACID: A NEW COMPOUND THAT POTENTIATES ACETYLCHOLINE EFFECT ON MUSCLE. P.A. Ferchmin, G. Escalona de Motta¶ and V.A. Eterović. Department of Biochemistry, Medical School, Univ. Central del Caribe and ¶Lab. Neurobiology, Medical Sciences Campus, University of Puerto Rico, Cayey, Puerto Rico, 00634.

A 1mM solution of succinic anhydride (SA) in 2-(tris(hydroxymethyl) methyl-amino)-1-ethanesulfonic acid (TES) potentiates acetylcholine-induced depolarization of frog sartorius endplate and acetylcholine-induced contraction of rectus abdominis muscle (Escalona de Motta and del Castillo, Nature 270:178-180, 1977). Succinic anhydride hydrolyzes rapidly in aqueous solutions and succinate is not effective. We report here the isolation of the active compound from SA-TES solution: The N-succinyl-TES, which we named castellic acid.

Castellic acid was isolated by paper chromatography (n-butanol: acetic acid: water, 100:22:50). It regenerated TES and succinate upon alkaline hydrolysis. The titration curve revealed a pKa near pH 5 (similar to the carboxyl groups of succinic acid) instead of the pKa=7.5 of the amine group in TES (which is converted to an amide in N-succinyl TES). The UV spectrum of castellic acid reveales its succinyl moiety with a maximum at 200 nm.

Castellic acid potentiates acetylcholine-induced contraction of frog rectus abdominis muscle at micromolar concentrations. Like the SA-TES solution, castellic acid is not an inhibitor of the acetylcholinesterase nor is it an agonist per se.

239.11 OPTICAL RECORDING OF NORMAL ELECTRICAL ACTIVITY FROM IDENTIFIED LEECH NEURONS MAINTAINED IN CULTURE. A.L. Obaid* and B.M. Salzberg Dept. of Physiology and Pharmacology, University of Pennsylvania School of Dental Medicine, Philadelphia, PA. 19104.

Identified leech neurons, isolated from segmental ganglia and encouraged to form synapses in culture (Ready and Nicholls, 1980), may provide a nervous system sufficiently simple to permit it's complete electrophysiological characterization. Optical methods for monitoring changes in membrane potential simultaneously from all of the elements of such a "nervous system", somata as well as the larger processes, could be used to study the responses to imposed stimuli, or the endogenous activity of networks constructed from identified elements. These preparations have favorable properties for multiple site optical recording and the advantage that their neuronal elements have been well studied in situ.

We have now established cultures containing individually excised P, N, and Retzius cells from segmental ganglia of the leech, Hirudo medicinalis, and maintained these preparations for up to 3 weeks. Action potentials recorded with microelectrodes were normal and characteristic of the cell type. NK 2367, a merocyanine-oxazolone dye that behaves as a linear potentiometric probe, was used to obtain optical recordings of action potentials from identified cultured leech neurons, and the optical signals were readily visible in a single sweep. Collagen coated tissue culture dishes containing groups of identified neurons were mounted on the stage of a modified Reichert Zetopan microscope and illuminated at 720± 15 nm, after having been incubated in a culture medium (L-15 plus 0.6% glucose, 10mM Hepes, 2% foetal calf serum, 10 ugm/ml Gentamycin) containing 200 ugm/ml NK 2367. A 32X long working distance objective, converted to water immersion, forms a real image of a cell or small group of cells in the objective image plane, first on a fine diffusion screen carrying the outline of a silicon photodiode array; then, after positioning the preparation, onto the array itself. The photocurrent outputs, representing the transmitted intensities reaching the individual elements of the array are passed in parallel to a multi-channel I-V converter and high gain amplifier, and the AC components of the signals are multiplexed into digital memory. The system is capable of recording optically from up to 128 sites simultaneously, using a PDP 11/34A computer and a Centronics 12X12 photodiode matrix array. We expect that this apparatus will be useful for analyzing the properties of small ensembles of identified leech neurons, and we hope that by monitoring all of the elements comprising a truly simple nervous system we will be able to follow the emergence of complex patterns of electrical activity from a relatively few synaptically coupled elements.

We are most grateful to John Nicholls and Mario Pellegrino for their generous assistance. Supported by USPHS grant NS 16824.

239.12 GENTAMICIN, AN AMINOGLYCOSIDE ANTIBIOTIC, DIMINISHES THE PRESYNAPTIC EFFECTS OF ACUTE CHOLINESTERASE INHIBITION. C. G. Carlson* and W-D. Dettbarn. Neuromuscular Dis. Res. Ctr., Vanderbilt Univ., Nashville, TN 37212.

The aminoglycoside antibiotics exhibit neuromuscular blocking activity at both the frog (Uchiyama et al., Eur. J. Pharm. 72: 271, 1981) and rat (Singh et al., Br. J. Anesthes. 50:109, 1978) neuromuscular junctions. This action is due primarily to a reduction in calcium dependent evoked transmitter release (Uchiyama et al., 1981; Singh et al., 1978). In the experiments reported here, the effect of the aminoglycoside gentamicin (GM) on spontaneous and evoked transmitter release was examined at the rat hemidiaphragm preparation. In addition, we also examined the effect of GM on the changes in nerve terminal excitability and spontaneous transmitter release induced by the irreversible cholinesterase (AChE) inhibitor paraoxon (Px); (Laskowski and Dettbarn, J.P.E.T. 210:269, 1979).

In control (normal AChE activity) preparations bathed in Krebs-Ringer solution, GM (625 μg/ml) reduced the average quantal content to values between 0 and 10 quanta per impulse at stimulation frequencies of 1 Hz. GM had no effect on the miniature endplate potential (MEPP) frequency in normal Krebs-Ringer solution (T = 30-32°C), but greatly diminished the increase in MEPP frequency produced by raising the potassium concentration to 15mM. The antibiotic also reduced the MEPP amplitude by 30 to 50%, but had no effect on the activity of AChE.

Preparations treated for 15 min with 5×10^{-6} M Px had reduced AChE activities (to 20 to 50% of control) and exhibited spontaneous muscle fasciculations and elevated MEPP frequencies. In these experiments, spontaneous antidromic firing (Randic and Straughan, J. Physiol. 173:130, 1974) was recorded differentially at the phrenic nerve (in vitro) with two suction electrodes. Following a 15 min exposure to Px, the rate of spontaneous firing was between .5 and 15 sec^{-1}. GM (625 μg/ml) reversibly diminished the rate of antidromic firing in these preparations, and essentially abolished the increase in MEPP frequency normally produced by Px.

The effects of GM on quantal content, presynaptic potassium sensitivity, and on the spontaneous antidromic firing induced by Px, are consistent with an action of the antibiotic on presynaptic calcium channels. The influence of GM in preventing the increase in MEPP frequency normally produced by Px suggests that the effects of Px on spontaneous release may be secondary to the increase in nerve terminal excitability induced by the inhibition of AChE.

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240.1 EFFERENTS ASCENDING FROM MIDBRAIN CENTRAL GRAY IN THE RAT REVEALED BY SENSITIVE USE OF TRITIATED AMINO ACID AUTORADIOGRAPHY. J. A. Eberhart, J. I. Morrell and D. W. Pfaff. The Rockefeller University, New York, NY 10021.

Although the midbrain central gray (CG) participates in the control of sexual behavior, nociception, and diverse other activities, projections from the rat CG have not been thoroughly described. We used tritiated amino acid autoradiography to reveal CG efferents to the telencephalon, diencephalon, rhombencephalon, and spinal cord. This report describes ascending projections.

Thirteen adult male and female Long-Evans rats received microinjections (10 nl, 170 μCi/μl) and 17 rats received iontophoretic injections (1-2 μa, 8-20 minutes, 20-35 μ tip diameter, 20 μCi/μl) of ^{3}H-leucine into, or lateral to, the CG at either the superior-, inter-, or inferior collicular level. Survival time was 2 or 3 days, and 6 μ sections from formalin-fixed, paraffin-embedded brains were exposed (NTB3 emulsion) for 1, 3, 5, or 21 months. Twenty-four additional animals received micro-injections (10 or 25 nl, 100 μCi/μl) of a cocktail of 5 ^{3}H-amino acids (leucine, proline, lysine, histidine, and tyrosine); these rats survived 6 or 10 days, and tissue was exposed for 3 months.

Ipsilateral to the injection site numerous fibers ascending from the CG sweep laterally, then ventromedially, coming to lie in the tegmentum dorsal to the medial lemniscus. Passing through the rostral tegmentum, fibers ascend in the relatively narrow region between the medial lemniscus and the substantia nigra. After passing rostrally through the ventral tegmental area of Tsai and the region dorsal to the mammillary body, they join the medial forebrain bundle to project throughout the lateral hypothalamic and preoptic regions. At the level of the ventromedial nucleus (which receives only a light projection) some fibers penetrate the dorsomedial nucleus, while others sweep laterally through the supraoptic commissure to reach the amygdala. More rostral projections include the ventral anterior hypothalamus, ventral preoptic area, and the area surrounding the fornix. There are labeled fibers in the diagonal bands and medial septum, and axo-somatic label in the lateral septum and nucleus accumbens. There is also label in the olfactory tubercle and a sparse projection to the frontal pole of the cortex. In addition to the ventral projection, the rostral CG is heavily labeled, with fibers and diffuse label invading the area just lateral to the CG. There are also projections to the pretectum, lateral geniculate body, midline thalamic nuclei, and labeled fibers in the stria terminalis. Labeled fibers passing through the collicular commissure augment a contralateral projection similar to, but less extensive than, that seen ipsilaterally.

(Supported by USPHS grant NSO6759.)

240.2 WHEAT GERM AGGLUTININ-GEL IMPLANTS IN THE MEDULLA AND SPINAL CORD OF THE RAT REVEAL LONG-PROJECTING DIENCEPHALIC NEURONS. M. Schwanzel-Fukuda*, J. I. Morrell and D. W. Pfaff. Rockefeller University, New York, NY 10021.

The importance of direct projections from hypothalamic and extra-hypothalamic diencephalic neurons in the mediation of behaviors and in the control of autonomic functions at medullary and spinal levels, together with the development of more sensitive retrograde tracing methods prompted us to investigate further the distribution and number of these long-projecting neurons. We delivered the lectin, wheat germ agglutinin (WGA) in a polyacrylamide gel pellet which permitted its slow release. Long Evans rats were unilaterally laminectomized in the cervical, lumbar or sacral regions of the spinal cord and a WGA-containing pellet was implanted following a small longitudinal incision in the cord. For study of projections to the medulla the atlanto-occipital membrane was cut and the WGA-containing gel implanted unilaterally. After a 2-5 day survival the rats were anesthetized, perfused with Bouins' solution, the tissues removed, blocked, post-fixed in Bouins' solution, and stored in 30% sucrose in PBS at 4°C. Frozen sections of 100 μm were cut and free-floating sections processed for immunocytochemical localization of retrogradely transported lectin. The retrogradely labeled neurons were visualized with antiserum to the lectin (A-WGA, E-Y Labs) and the unlabeled peroxidase antiperoxidase method. The cytoplasm of the retrogradely labeled cells was filled with dark brown granular reaction product and frequently two or more processes were visible, the nuclei were clear. No retrogradely labeled cells were detected in the brains of control animals with pellets under dura adjacent to unincised cords, indicating that WGA was not taken up by intact axons or transported in the blood or cerebrospinal fluid. Controls for specificity of A-WGA showed no cross-reactivity with any endogenous brain substance. Absorption of A-WGA with WGA eliminated all positive reaction.

Our data revealed many more diencephalic neurons project to medulla and spinal cord than have been described before. Retrogradely labeled neurons were most numerous in specific subdivisions of paraventricular nucleus, caudal lateral hypothalamus and zona incerta, majority ipsilateral. Labeled cells were also seen in nucleus of the anterior commissure, anterior hypothalamus, medial basal hypothalamus including arcuate nucleus and the region ventral to the ventromedial nucleus (VM), and in the posterior hypothalamic area medial to the mammillothalamic tract extending caudally to the ventral and medial aspects of the fasciculus retroflexus. The preoptic area and VM were virtually without labeled neurons. In addition large numbers of labeled neurons were visible in midbrain after implant in medulla or cord, and in amygdala after implant in the medulla.

240.3 COLLATERALIZATION OF SUBICULAR CORTEX AXONS TO DIENCEPHALIC AND TELENCEPHALIC TARGETS. J.M. Wyss, M.K. Donovan, K. Sripanidkulchai and B. Sripanidkulchai*. Dept. of Anatomy, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Swanson et al. (1981)* have recently demonstrated that the axons arising from Ammon's horn neurons collateralize extensively so as to project to multiple telencephalic target sites. In their initial inspection of the subiculum, these authors observed a projection pattern with only one-third of the neurons in most portions of the subiculum exhibiting collateralization to the septum and entorhinal cortex. The present study has used the fluorescent dye tracing technique in order to determine the exact location of neuronal somata within the subicular cortex which project to the diencephalon, telencephalon (entorhinal cortex), or to both via axonal collaterals. In 35 experiments, an albino rat received an initial true blue (Illing, West Germany) injection, into either the entorhinal cortex or a diencephalic terminal field, and 2 days later the same animal was stereotaxically injected with nuclear yellow (Hoechst, West Germany) in the alternative field. After appropriate survival, the animals were reanesthetized, perfused and frozen serial sections were cut through the subicular cortex. These sections were mounted on clean, uncoated slides and examined with a Leitz A filter cube system. The resulting data demonstrate that the greatest collateralization to the two sites arises from the neurons of the subiculum proper. In this region approximately one-third of all neurons project to both the entorhinal cortex and the hypothalamus (either the mammillary bodies or the ventral medial hypothalamic nucleus). The hypothalamic and cortical projection cell bodies in this region are intermingled extensively with each other. In the cytoarchitectonically more organized regions of the subicular cortex, i.e. the pre-, para- and post-subiculum, the situation is quite different. In these areas neurons project to the hypothalamus or entorhinal cortex but very seldom does a single neuron project to both areas, and the neuronal somata are spatially segregated according to their projections. The entorhinal cortex projecting somata are located in the superficial neuronal cell layer whereas the hypothalamic projecting neurons are more deeply placed. The somata projecting to the thalamus are the most deeply located neurons in all regions of the subicular cortex, and extremely few of these collateralize to the entorhinal cortex.

*Swanson, LW, Sawchenko, PE and Cowan, WM (1981) J. Neurosci. 1:548-559.

240.4 GENETIC SELECTION FOR EXPLORATORY PATTERNS IN RATS LEADS TO DIFFERENCES IN HIPPOCAMPAL CIRCUITRY. H.-P. Lipp* and H. Schwegler. (SPON: L. Adelman). Dept. of Psychol. & Brain Sci., Mass. Institute of Technology, Cambridge, MA 02139 and Inst. of Hum. Genet., Univ. Heidelberg, D-69 Heidelberg, FRG.

Genetic and experimental variations in the percentage of intra- and infrapyramidal hippocampal mossy fiber terminals (IIP-MF) in mice and rats relate closely to acquisition and performance of two-way avoidance; the more IIP-MF, the poorer shuttle-box learning (Schwegler and Lipp, Behav. Brain Res., in press; Lipp et al., Neuroscience Letters Suppl. 7: 46, 1981). This task, however, involves several factors. In a search for psychological variables actually related to IIP-MF, we undertook an allometric study of hippocampal terminal fields in two rat strains selectively bred for high or low exploration scores (Naples High Excitable and Low Excitable Rats, NHE, NLE: Sadile et al., Abh. Akad. Wiss. DDR 5: 203-218, 1979). Planimetric analysis was done one 5 Timm-stained horizontal sections per rat, taken from the mid-septotemporal level (3 NHE, 4 NLE) according to a standardized procedure (see refs). The low-exploratory rats had significantly more mossy fibers synapsing on basal dendrites of hippocampal pyramidal neurons (IIP-MF, $p < 0.005$ two-tailed). Less distinct (and probably less reliable) differences were found for the relative sizes of the following hippocampal fields: the medial entorhinal projection to area dentata (larger in NLE, $p < 0.025$); stratum oriens (larger in NLE, $p < 0.05$), suprapyramidal mossy fiber layer (smaller in NLE, $p < 0.05$), and stratum pyramidale (smaller in NLE, $p < 0.05$). Apparently, selection for low exploratory behavior has led to an enlarged IIP-MF projection. We have shown elsewhere that variations in IIP-MF do not relate to variations in freezing behavior. The present data are compatible with the idea that the amount of IIP-MF correlates positively with a tendency to persist in ongoing behavioral pattern under conditions of stress. This might account for behavioral stereotypes, poor shuttle-box learning, and reduced exploratory behavior. Supp. by SNF 3.516 and DFG. This study was done at the Inst. of Anatomy, Univ. Lausanne, Switzerland; the rats were a gift from Dr. Sadile, Inst. of Physiology, Univ. of Naples, Italy.

240.5 COMMISSURAL AXONS SYNAPSE WITH IDENTIFIED BASKET CELLS IN THE RAT DENTATE GYRUS: A COMBINED DEGENERATION - GOLGI -ELECTRON MICROSCOPIC STUDY. C.E. Ribak and L. Seress*. Department of Anatomy, University of California, Irvine, CA. 92717.

Four varieties of basket cells have been identified in previous light microscopic studies of the hippocampal dentate gyrus. The somata of basket cells are located either subjacent to, or within the granule cell layer and their size is considerably larger than that of granule cells. All basket cells have an axonal plexus which distributes in the granule cell layer and forms a veil about the somata and proximal dendrites of granule cells. Using a combined Golgi-electron microscopic method that utilizes gold toning of silver impregnated neurons, we recently demonstrated that the axons of basket cells form axosomatic and axodendritic symmetric synapses with granule cells. Although rarely observed, the axon of a basket cell may synapse with its own apical dendrite in a way similar to the autapse described for aspinous stellate cells in the neocortex. Since the smooth apical dendrites of basket cells ramify in the molecular layer as do the spinous dendrites of granule cells, they would be in a position to receive the same afferent systems that synapse with the granule cells. This study was undertaken to determine if one such afferent system, the commissural axons which arise from the contralateral hilus, forms synaptic contacts with basket cells.

Rats with lesions of the hippocampal commissure were perfused 48 hrs after surgery. Sections which were contralateral to the lesion and contained Golgi-stained basket cells were obtained from their brains, gold-toned, and processed for electron microscopy. Degenerating axon terminals were observed mainly in the inner third of the molecular layer as well as in the granule cell layer and in that part of the hilus 50-70 microns beneath the granule cell layer. These terminals formed both asymmetric and symmetric synapses which are associated with excitatory and inhibitory function, respectively. Thus, these results are consistent with recent physiological and immunocytochemical data that indicate this presumed excitatory pathway also contains some inhibitory GABAergic axons. The degenerating terminals of commissural axons were found to synapse with the apical dendrites and somata of identified basket cells. In addition, these axons made synapses with granule cell dendrites. Therefore, the commissural pathway directly contacts granule and basket cells in the rat dentate gyrus. This finding indicates that both the projection and local circuit neurons of the dentate gyrus are contacted by the same afferent system.

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240.6 A COMPARISON OF AMYGDALOID AND HIPPOCAMPAL PROJECTIONS TO THE THALAMUS IN MONKEYS J. P. Aggleton* and M. Mishkin. (SPON: R. Desimone). Lab. of Neuropsychology, NIMH, Bethesda, MD 20205.

Clinical and experimental evidence indicates that damage either to medial temporal or medial thalamic structures may result in anterograde amnesia. This evidence, which suggests that the two regions form a system essential for normal memory, prompted us to reinvestigate the direct anatomical connections between them. Projections from the hippocampus and amygdala to the thalamus were studied in cynomolgus monkeys (Macaca fascicularis) with both anterograde and retrograde tracers. Horseradish peroxidase (Sigma VI, 40% solution) was injected (0.15-0.22 uL) into the medial thalamus in four monkeys and an iontophoretic injection was placed in nucleus medialis dorsalis (MD) in a fifth; the sections were subsequently reacted with tetramethyl benzidene. Unilateral injections of a mixture of [^{3}H]-proline and [^{3}H]-leucine were placed in the amygdala in seven monkeys and in the hippocampus in five.

Although projections to the thalamus were found to arise throughout the amygdala, the basal group, and particularly the basomedial nucleus, provides the greatest input. The amygdalofugal fibers sweep through the substantia innominata and then travel in the inferior thalamic peduncle to enter the head of the thalamus before passing caudally to reach the magnocellular portion of MD where they terminate in a crude topography. Allocortical areas adjacent to the amygdala also contribute significantly to this projection.

Hippocampal projections to the thalamus arise predominantly in the subiculcum and terminate most heavily in nuclei anterior medialis, anterior ventralis, and lateralis dorsalis. Lighter projections to nuclei reuniens, centralis latocellularis, rotundis, and paraventricularis were also observed. All of these projections course through the medial fornix, though nucleus lateralis dorsalis receives an additional, nonfornical input from the hippocampus which runs through the pulvinar.

These findings indicate that the direct projections of the amygdala and hippocampus to the thalamus travel by distinct routes and terminate in separate regions with little, if any, overlap. This pattern of projections suggests that the limbo-thalamic memory system consists of two parallel circuits dominated by an amygdalo-medialis dorsalis pathway and a hippocampo-anterior nuclei pathway, respectively.

240.7 SEPTAL INPUTS TO VASOPRESSIN (VP) NEURONS: A LIGHT (LM) AND ELECTRON MICROSCOPIC (EM) ANALYSIS. B. J. Oldfield*, A. Hou-Yu* and A.J. Silverman. (SPON: C. R. Noback) Depts. of Anat./Cell Biol. and Neurol., Columbia Univ., P&S, New York, N.Y. 10032.

VP neurons within the paraventricular nucleus (PVN) are involved in the regulation of neuroendocrine and autonomic functions. It has been shown in several recent studies that VP cells are influenced by circulating glucocorticoids (GC). It is unclear how this action is mediated as PVN cells have few if any GC receptors. It has been suggested that the PVN receives a synaptic input from limbic structures that contain neurons capable of concentrating GC. It is possible that the metabolic effects of GC on VP cells are mediated via this synaptic input. The aim of the present study is to characterize the afferent input from one of these regions, the septum (S), to immunocytochemically identified VP neurons in the hypothalamus. Anterograde transport of HRP has been utilized to trace afferent pathways and a double labeling paradigm has been derived where by anterogradely transported HRP and VP immunoreactivity can be visualized in the same EM sections. HRP was iontophoresed from micropipettes into the lateral S of adult Long-Evans rats.After 24 hr, rats were perfused with either 2% glutaraldehyde or a mixture of 1% paraformaldehyde, 2.5% glutaraldehyde. LM HRP histochemistry was carried out on 50 um vibratome sections using TMB. For EM, sections were first exposed to a monoclonal antibody to VP (III D-7) followed by a second antibody conjugated to HRP. Tissue was then preincubated in 0.5% $CoCl_2$ and then incubated in DAB with H_2O_2 generated by the glucose oxidase method. These sections were osmicated and embedded in Epon. HRP injections were small and centered within the dorsal, intermediate and ventral parts of the lateral S. In a few cases, injections were situated more medially, overlapping the medial S. HRP labeled fibers arising from the S extended ventrally and rostrally through the medial preoptic area and lateral hypothalamus to the anterior hypothalamus. Following lateral S injections, labeled fibers appeared to terminate in the vicinity of the PVN, SON and SCN forming discrete shells around the boundaries of each nucleus. Appreciable numbers of labeled terminals were only present within the confines of the PVN when injections involved the medial S. EM observations have confirmed that the HRP labeled structures seen in the LM surrounding the PVN are preterminal and terminal axons. In addition, both HRP reactivity and VP immunoreactivity could be visualized simultaneously in these thin sections. As yet synaptic associations between S terminals and VP positive neurons have not yet been observed but it is hoped that the compatibility of EM HRP histochemistry and VP immunocytochemistry will make it possible to describe the afferent input to VP cells. Supported by AM 20337.

240.8 A CORRELATIVE GOLGI AND HRP STUDY OF NEURONS IN THE BASOLATERAL AMYGDALA OF THE RAT. A.J. McDonald* (SPON: J. Kosh). Dept. of Anatomy, University of South Carolina, School of Medicine, Columbia, South Carolina 29208.

Analyses of 51 rat brains impregnated with the Golgi-Kopsch or rapid Golgi techniques reveal that the basolateral nucleus of the amygdala (ABL) contains large, spiny cells (class I neurons) that appear to be projection neurons, and two types of spine-sparse cells (class II and class III neurons) that display dense local axonal arborizations. Class I neurons have large pyramidal cell bodies (15-22 µm) while those of spine-sparse neurons tend to be smaller (9-17 µm) and more ovoid. Based on axonal morphology it appears that class I cells are projection neurons while spine-sparse cells are local circuit neurons. This hypothesis has been tested by injecting HRP (Sigma, type VI) into areas that receive projections from ABL and comparing sizes of labeled and unlabeled perikarya in ABL. Injections (.05-0.2 µl of 30-40% HRP) were made into frontal cortex (FC), ventral striatum (VS) and bed nucleus of the stria terminalis (BST) using a 5.0 µl microsyringe. After a 24 hr survival time the animals were perfused and the brains were processed according to the TMB proceedure of Mesulam. Sections were counterstained with Pyronin-Y. All perikarya, both labeled and unlabeled, in various portions of ABL were drawn with the aid of a camera lucida and measured using a calibrated rule. Retrogradely-labeled neurons were always large cells, most of which were pyramidal in shape. A large injection that involved BST and VS left only 7% of the cells in the posterior division of ABL unlabeled; these were all small ovoid neurons. Injections into FC or VS labeled more than half of the cells in the anterior division of ABL; these neurons all had large pyramidal cell bodies. Both large and small neurons were unlabeled. These results suggest that only class I neurons of ABL project to rostral forebrain regions. Work is currently in progress to determine if class II and class III neurons are involved in internuclear or additional extrinsic connections of ABL.

240.9 ELECTROPHYSIOLOGICAL EVIDENCE FOR CENTRAL AND PERIPHERAL CONNECTIONS WITH THE AMYGDALA IN AWAKE CATS. M. M. Knuepfer, A. Eismann*, H. Stumpf* and G. Stock*. Dept. of Physiology, University of Heidelberg, 6900 Heidelberg, F.R.G.

The central nucleus of the amygdala (AC) has been implicated in the integration of autonomic responses to arousal and stress. Though other studies have described several central connections with the AC including projections from substantia nigra (SN) and locus coeruleus (LC), the role of these pathways is unknown. This study was designed to examine responses of single units in the AC to baroreceptor and arousing stimuli and to electrical stimulation of central sites in unanesthetized cats. Cats were prepared by exposing the dura and cementing a brass socket directly over the AC to allow access to the brain. Bipolar electrodes were also implanted in the LC, the SN and, in some cases, the contralateral AC. Arterial pressure was measured from a carotid arterial cannula and drugs were administered through a jugular cannula. Cats were tested weekly for one to four months after recovery. Metal microelectrodes (9-12 MΩ) were lowered into the AC using a miniature, light-weight motor mounted to the socket in awake, unrestrained cats. Of all spontaneously active units identified, 37 of these were histologically verified to be in the AC. Sixty-two percent (21 of 34 cells) were responsive to SN stimulation with a greater number of cells responsive to ipsilateral (13/16) compared to contralateral (8/18 stimulation. Stimulation of the LC produced a response in 55% of cells tested with a majority of responsive units exhibiting a decrease in activity. Four of nine units were excited by stimulation of the contralateral AC. These data support neuroanatomical evidence for connections of the AC with the contralateral AC, the SN and the LC. Seventeen of the spontaneously active units responded to an increase in arterial pressure produced by a bolus of phenylephrine (10ug in 0.1ml, iv). Of these cells, nine were characterized by an inhibition of spontaneous activity. These data support work by our laboratory and others demonstrating in anesthetized cats that baroreceptor afferent nerves project to the AC. Finally, the majority of units were responsive to various stimuli affecting the level of arousal. These included units which were responsive to acoustic stimulation (25/37) and to general arousal elicited by tactile, visual or olfactory stimuli. In addition, several units were identified that were responsive to more than one stimulus suggesting a convergence of separate projections at this level. These results identify both central and peripheral projections to the AC and their integration at this level. Furthermore, using unanesthetized cats enabled us to demonstrate the effect of stimuli causing arousal on amygdaloid unit activity.

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240.10 THE LIMBIC SYSTEM AND SALT APPETITE IN RATS. A. Derick Dalhouse, P. Turner*, and O. Brooks*. Department of Psychology, Jackson State University, Jackson, MS 39217.

Septal lesions have been reported to produce increases in sodium chloride (NaCl) intake in rats while amygdaloid lesions have been associated with both increases and decreases in NaCl intake in rats. In order to determine if these two limbic areas exert reciprocal control over NaCl intake in rats as they have been reported to over such behaviors as social cohesiveness, sensitivity to foot shock, and hyperreactivity, 90 to 120 day old Sprague Dawley Male Albino Rats were subjected to successive septal and amygdaloid (S/A) successive amygdaloid and septal (A/S) or simultaneous septal and amygdaloid (S&A) lesions. They were placed ad lib on 3% NaCl, distilled water and sodium free food (ICN Pharmaceutical). Intake data are collected on 6 consecutive days prior to the first lesion, used as base-rate data, and to counterbalance the groups. Six days of intake data was collected after the first and second lesions following 7 days post operative recovery.

Amygdaloid lesions increased NaCl intake in both the A/S ($p < .01$) and S/A ($p < .05$) rats while septal lesions decreased their NaCl intake ($p < .01$ & $p < .05$ respectively). The S&A group as well as the sham operated controls, showed no significant pre-post operative differences in their NaCl intake.

These results suggest that the septal and amygdaloid nuclei may infact exert reciprocal control over NaCl intake in rats. However, in this experiment animals with septal lesions showed a decrease in their NaCl intake, the opposite of findings reported in the literature. We are currently attempting to replicate our results to ascertain that our findings are replicable.

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241.1 CHANGES IN THE HUMAN POSTURAL RESPONSE SYSTEM WITH AGING. M.H. Woollacott, A. Shumway-Cook* and L.M. Nashner. Dept. of P.E., University of Oregon, Eugene, OR 97403 and Good Samaritan Hospital, Portland, OR 97209.

Coordination of muscle activity in posture responses of 12 elderly (61-78 yrs.) subjects were compared to those of young (19-38 yrs.) adults using a moveable platform with separate supports for each foot and recording the EMG activity of four leg muscles (gastrocnemius (G), tibialis anterior (TA), hamstrings (H), and quadriceps (Q). Previous work on young adults shows that unexpected anterior or posterior platform movements produce body sway in the opposite direction and elicit long latency responses in the proximal and distal musculature with a stereotypical organization (muscles in the lower leg respond at 100 ms, followed 10-20 ms later by those in the upper leg). Comparison of long latency responses in aging versus young adults has shown a breakdown in the muscle coordination mechanisms which structure the temporal and spatial parameters of muscle actions. There is a slight but significant increase in the latency of the long latency responses. For posterior sway perturbations the TA latency was 109±9 ms for the aging group compared to 102±6 ms for the young adults. There also appears to be a secondary breakdown in synergic firing patterns for upper and lower leg muscles in many aging adults. Thus the Q muscle, which normally responds 10-20 ms later than the TA muscle, now responds either very late (154±15 ms as compared to 122±13 ms for normals) or in some aging subjects, responds in advance of the TA. Finally, there appears to be instability in the metrics of activation of the synergistic muscles. In young adults correlations of EMG amplitudes of proximal and distal muscles of the synergy are high ($\bar{r}$=.972) with little intersubject variability, whereas correlations in older subjects are significantly lower ($\bar{r}$=.762; $p<.01$), with significantly greater intersubject variability ($p<.01$).

Rotational perturbations, causing direct ankle rotation, but little sway, produce muscle response synergies identical to those produced by antero-posterior displacements. But in this context, the muscle response synergies are destabilizing. In normal adults the responses attenuate over 3-5 trials and secondary compensatory vestibular responses return the center of mass to normal before the limit of stability is reached. In the aging subjects, 50% fell on the first trial, suggesting an increased reliance on the long latency response system. Compensatory responses did not appear to have sufficient force to stabilize the center of mass with sufficient speed and power to prevent a fall. However, only one aging subject fell on subsequent trials. All others normally adapted the synergic responses. This suggests that processes underlying the function of adaptability of postural responses are still normal in the population of older adults studied here.

241.2 PARENTAL AGE AS A RISK FACTOR IN ALZHEIMER'S DISEASE. Suzanne Corkin and John H. Growdon*. Dept. Psychol., Mass. Inst. Tech., Cambridge, MA, 02139; Mass. Gen. Hosp., Boston, MA 02114.

Many patients with Down's syndrome develop Alzheimer's disease in middle age, and patients with presenile dementia (onset before age 65) have a significantly greater number of relatives with Down's syndrome than occurs in the general population. Advanced parental age may be a risk factor for Down's syndrome and is a postulated risk factor for Alzheimer's disease. It was therefore of interest to obtain parental age data for a group of patients with Alzheimer's disease (N = 36) and an age-matched group of healthy people (N = 34). These groups were selected according to research criteria and were from the same socioeconomic levels. At the time of the Alzheimer patients' birth, mean maternal age was 27.7 years, and mean paternal age was 30.8 years. The comparable mean ages for the mothers and fathers of the healthy subjects were 27.4 years and 29.4 years. These ages did not differ significantly from those for the Alzheimer group. The mothers of the Alzheimer patients died at a mean age of 75.6 years, and the fathers at a mean age of 70.3 years. The mothers and fathers of the healthy subjects died at 74.4 years and 71.1. years, respectively. In a further analysis, patients with Alzheimer's disease were divided into two groups on the basis of age at onset of disease. Parental age data did not distinguish those whose age at onset was less than 65 years from those whose age at onset was 65 years or over. The values for both Alzheimer groups were comparable to those for the healthy group.

We conclude that giving birth in middle age does not place offspring at greater risk of later having Alzheimer's disease.

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241.3 CONDITIONED LEARNING DEFICITS IN AGED CATS. J.Harrison, J.Buchwald, and L.Butcher, Depts. of Physiol. and Psychol., BRI and MRRC, UCLA Med. Cntr., L.A., Ca. 90024

The classically conditioned eyeblink response is a robust and stereotypical response for which a large body of normative parametric data is available. In earlier work, our laboratory showed that this conditioned response could be acquired and discriminated by cats after bilateral hemispherectomy and even after midbrain decerebration. These and other data have emphasized the importance of the brainstem reticular formation in the acquisition of this simple learned response. In an ongoing study of aging, aged cats (10-23 years) were subjected to classical eyeblink conditioned procedures. As in our earlier studies, the tonal CS was delivered free field and reinforced with a brief shock to the outer canthus (US) to produce a brisk unconditioned blink response; unreinforced click stimuli were randomly interspersed among the paired CS/US trials. The CS, a 1.5 sec tone, was sufficiently loud to produce well defined auditory brainstem responses in all subjects. Conditioning was carried out on restrained awake subjects in a sound isolation room. Eyeblinks were observed through a one-way mirror with concurrent EMG recordings from the orbicularis oculi. The 25 CS/US trials were randomly interspersed among 475 click trials. The inter-trial interval was variable with a mean of 30 sec. Six control cats (1-3 years) showed an 80-100% conditioned eyeblink response level at a mean of 388 CS/US trials. Of 11 aged cats (10-23 years) subjected to the same training, 8 cats showed no conditioned eyeblink response (CR) after 1000 CS/US trials, when training was terminated; only 3 aged cats acquired the CR, at a mean of 525 CS/US trials. To investigate this learning deficit further, the protocol was simplified: the inter-trial interval was shortened, the clicks deleted and the CS duration was shortened from 1500 to 800 ms. Thusfar, 2 of 3 aged cats tested have not acquired the CR with this protocol. The one cat which did acquire a CR, did not then generalize this CR to the same CS in the prior more difficult training protocol. From these data we infer that acquisition of a classically conditioned eyeblink response is markedly abnormal in some aged cats. Based on previous studies relating acquisition of this CR to the brainstem reticular formation, abnormal associative functioning of the brainstem reticular formation in aged cats is suggested. Neurons identified as cholinergic on the basis of intense AChE staining and ChAT biochemistry have been demonstrated throughout the reticular formation of young rats and cats. A histochemical brain map of the non-learning old cats is being constructed to test the hypothesis that cholinergic deficits in the reticular core of the old cat brain are significantly correlated with deficits in conditioned eyeblink learning.

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241.4 NEOCORTICAL CHOLINERGIC DEFICIT AND BEHAVIORAL IMPAIRMENT PRODUCED BY SUBCORTICAL NEUROTOXIC LESIONS. B.Lerer* and E.Friedman* (SPON: S.H. Ferris). Departments of Psychiatry and Pharmacology, New York University Medical Center, New York, New York, 10016.

Neurons whose cell bodies lie ventral to the globus pallidus, in the area of the nucleus Basalis of Meynert, provide extensive cholinergic innervation to fronto-parietal cortex. Destruction of these neurons causes a marked depletion of cortical choline acetyltransferase (CAT), the synthetic enzyme used as a marker for acetylcholine neurons. We wish to report that destruction of the basalis-cortex pathway also produces behavioral deficits in the rat suggestive of memory impairment.

Bilateral microinjections of the neurotoxin kainic acid, which specifically destroys cell bodies, were placed stereotactically in the area of the nucleus Basalis of young, male Sprague-Dawley rats. The resulting lesions reduced CAT levels in fronto-parietal cortex by about 35% in the lesioned group as compared to a control group that received injections of the vehicle buffer only. In striatum and hippocampus, CAT levels remained unaffected.

For several days following surgery, lesioned rats were aphagic and adipsic and they displayed motor and reflex abnormalities. During this recovery period animals were maintained via intubation of liquid infant formula. Behavioral testing was conducted 10-14 days post-operatively after normal ingestion, grooming and locomotion had resumed. Lesioned rats showed significant impairment in 24 hr retention of a step-through passive avoidance task. Motor activity rates and shock sensitivity were not significantly different in lesioned animals as compared to controls, and therefore do not account for the retention deficit.

A second group of rats was run daily for 2 weeks before surgery in an unbaited Y-maze spontaneous alternation task. Pre-operatively, all rats consistently alternated in successive choices of maze arms; post-operatively, lesioned rats perseverated significantly in their choices.

Many animal and human studies point to the role of the cholinergic system in learning and memory. Indeed, the memory impairments and reduced cognitive functioning characteristic of senile dementia of the Alzheimer type (SDAT) have been correlated with reduced cortical CAT in post-mortem human tissue. Our data show that when this neurochemical aspect of SDAT is induced in young rats, behavioral impairments suggesting memory dysfunction result. This paradigm should prove useful as an animal model for studying SDAT.

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241.5 THE EFFECT OF AGE ON THE LEVELS OF 5-HYDROXYTRYPTAMINE AND 5-HYDROXYINDOLE ACETIC ACID IN DISCRETE REGIONS OF RAT CNS. Larry J. Embree, Isaac F. Roubein, David W. Jackson* and Danny Kay*. VA Med. Ctr., and Dept. of Neurology, LSUMC-Shreveport, LA 71130.

Evidence in the literature indicates that 5-hydroxytryptamine (5-HT) is involved in a variety of neurological problems such as cerebral ischemia, trauma, vasospasm, stroke, migraine and mood disturbances. Investigations on 5-HT metabolism in mammalian brain have been less extensive than those on catecholamines, and there have been limited and somewhat conflicting reports on the effect of age on the concentrations of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in discrete regions of rat CNS. Therefore, we have measured the levels of 5-HT and 5-HIAA in six CNS regions from aged rats to determine if regional differences occur in the levels of 5-HT and 5-HIAA with aging. Male Sprague-Dawley rats, 3-4 months and 26-29 months old, were used in this study. The animals were sacrificed by decapitation and the following regions were dissected from the CNS: striatum (S), hypothalmus (HT), midbrain (MB), pons-medulla (P&M), pineal gland (P) and cervical spinal cord (CSC). Levels of 5-HT and 5-HIAA were determined using HPLC with amperometric detection. After homogenizing the tissue in 0.4M $HClO_4$, the pellet was resolubilized and protein level was determined by the Lowry method.

	5-HT nanogram/mg protein		5-HIAA nanogram/mg protein	
	young	old	young	old
MB (9)	7.22 ± 0.66 (9)	6.19 ± 0.78	8.93 ± 0.43	9.47 ± 0.54
P&M(9)	5.48 ± 0.22 (9)	3.84 ± 0.46	5.46 ± 0.34	5.60 ± 0.50
S (8)	27.97 ± 2.26 (8)	25.45 ± 1.13	6.08 ± 0.62	6.51 ± 0.46
HT (8)	7.69 ± 0.79 (8)	7.86 ± 1.11	9.69 ± 0.55	9.63 ± 0.48
CSC(7)	4.78 ± 0.61 (9)	2.86 ± 0.24	3.00 ± 0.36	2.91 ± 0.38
P (8)	685.10 ±64.90 (6)	613.47 ±90.06	80.28 ± 8.28	87.26 ±18.82

These data show a decrease in the level of 5-HT in five out of six CNS regions examined from aged rats. This decrease was significant in pons-medulla and cervical spinal cord ($P<0.05$ by repetitive Student's *t*-test) and likely reflects an age-related change(s) in 5-HT metabolism in these two regions. There was no significant change in the level of 5-HIAA in any of the six regions examined.

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241.6 BEHAVIORAL RESPONSE AND BRAIN LEVELS OF METHADONE: DIFFERENCES IN YOUNG AND AGED MICE. L.D. Middaugh and I.M. Kapetanovic.* GRC, National Institute on Aging. Baltimore, MD 21224

Several changes occur in aged humans and laboratory animals which alter their response to narcotic analgesics. Although opiate receptors are reduced in aged animals, the behavioral consequences of this reduction and the age related changes in the pharmacokinetics of methadone are not well studied. In the present study, plasma and brain levels of methadone produced by subcutaneous injections of the chloride salt (2.5 mg., 7.5 mg., 15.0 mg. or 22.5 mg/kg) and resultant change in locomotion were determined in young (6-8 mos.) and aged (30-32 mos.) C57 BL/6 mice.

In the first experiment, activity of habituated mice in either age group was uninfluenced by either saline or the low methadone dose. At the higher doses, activity rapidly increased, then declined over a three hour test period. Time to maximum elevation was similar for the two age groups. However, activity of the aged mice was elevated less extensively but for a longer duration than that of young mice.

In the second experiment, locomotor activity and methadone levels were determined in groups of mice at several intervals after the highest dose. Young mice injected with drug were more active than the aged during the first two intervals; however, the degree of elevation above their respective saline congroups was similar. At later time periods young mice were less active than the aged; and at two hours, activity was reduced and elevated for young and aged mice respectively. Methadone levels were similar across time for both age groups in plasma and for the first two time points in brain. Aged mice, however, had higher brain drug levels than younger mice beginning one hour post-injection.

In the final experiment, both age groups were injected with the three highest doses. Activity and methadone concentrations were determined at one and three hours post-injection. Within each time period, activity was inversely related to dose and brain levels of methadone for both age groups. The decline in activity with dose increase or across time was greater for young than for aged mice in spite of higher brain levels of the drug in the latter. Plasma levels were again similar for the two age groups for all doses and times.

The results indicate that the methadone doses used elevate then depress locomotion of young C57 mice and that there is an inverse relationship between locomotion and drug levels in brain. Aged animals are less responsive to the drug which cannot be accounted for by age related changes in drug absorption, transport or metabolism. Although untested, the behavioral results are compatible with age related differences in brain distribution of the drug or a reduction in opiate receptors.

241.7 SPONTANEOUS ACTIVITIES IN AGED RATS: RESPONSE TO PERGOLIDE OR AMPHETAMINE AS MEASURED IN AUTOMATED ACTIVITY CHAMBERS. M. J. Schmidt, D. V. Pearson,* D. L. Hymson,* and M. D. Hynes, Lilly Research Labs., Eli Lilly and Company, Indianapolis, IN 46285.

Spontaneous and drug-induced motor behavior was studied in rats to quantify senescence-related changes in dopaminergic brain function, longitudinally and non-invasively. Omnitech Digiscan optical activity monitors were used to automatically record parameters of motor activity. Parameters measured include horizontal activity, distance traveled, number of movements, stereotypic behavior and time spent at rest.

Spontaneous activities were monitored in male Fisher 344 rats 7 or 29 months of age. Young rats had locomotor activity levels greater than those of old rats, as indicated by increases in horizontal photocell beams broken (+56%) and total distance traveled (+77%). No difference between the two age groups was detected in measurements of stereotypic behavior (number of repetitive movements and time engaged in this behavior) or the number of movements made. The 7- and 29-month old rats were subsequently placed into one of two groups and given either vehicle or 0.5 mg/kg of pergolide, a direct-acting dopamine agonist. Activity was increased approximately 75% by pergolide in both age groups. Pergolide also increased the distance traversed and the number of movements made, but age differences were not apparent. Stereotypic behavior was not affected by pergolide.

One week after the pergolide experiments, spontaneous activity was again measured. There was evidence of behavioral adaptation in both age groups during the second week of testing. Compared to week 1, the 7-month-old rats had a 71% decrease in spontaneous locomotor activity, whereas the 29-month-old rats exhibited only a 45% reduction. All other parameters were also decreased in both age groups during the second week. Following assessment of spontaneous locomotor activity, these rats were treated with either saline or 1 mg/kg amphetamine, a dopamine releasing drug. Horizontal activity was increased 6-fold in both age groups in response to amphetamine. The distance traversed was elevated 8-fold in both age groups. Stereotypic behavior was elevated by amphetamine 4-fold in old rats, and 5-fold in young animals, but the total number of movements was increased to the same degree in both ages groups.

These results indicate that while the levels of some spontaneous motor activities change with age, not all behaviors are age-sensitive. Furthermore, dopaminergic stimulation elicits the same amount of behavioral changes in both age groups, suggesting that the biochemical changes observed in the dopamine system of rodents during senescence may be of minimal functional consequence. The study also illustrates the utility of automated systems for monitoring behavior.

241.8 INTRACELLULAR RECORDINGS FROM NEURONS OF THE MOTOR CORTEX OF OLD CATS. C.D. Woody and E. Gruen*. Depts. of Anatomy and Psychiatry, UCLA Medical Center, Los Angeles, CA 90024.

Intracellular recordings were made from 201 neurons of the motor cortex of five cats ranging in age from 10 to 23 years. Methods of recording from the unanesthetized awake preparations were as described earlier (Woody and Black-Cleworth, J. Neurophysiol., 1973). The resting potentials of the cells averaged 52 ± 11mV (SD) with action potentials of up to 70mV. Resting potentials in 65 cells from 3 control cats of 1.5 to 2.5 years of age averaged 53 ± 9mV. An average rate of discharge of 27 spikes per sec was recorded in the cells in old cats versus a rate of 20 spikes per sec in the cells from the control animals. The difference in the means per animal was significant at $P<.05$ (27 ± 5 versus 20 ± 3). Depolarizing ramp currents were passed intracellularly to test accommodative properties and possible deterioration of the membranes of the cells. Previous studies (Schlue et al, J. Neurophysiol., 1974) had shown that large currents were required to discharge cells with damaged membranes when the currents were applied with a slowly rising ramp. This effect is termed a minimal gradient (MG) response. Accommodation may be reflected by a much smaller increase in depolarizing current needed to discharge the cell as the slope of the ramp decreases. This effect is termed a ceiling (C) response. An increased rate of firing to a fixed level of current irrespective of the slope of the ramp is termed a simple (S) response. Of 90 cells injected with ramp currents in unanesthetized older animals, 69 showed S responses, 19 C responses, and 2 MG responses. Of 38 cells injected in control animals, 34 showed S responses and 4 showed C responses. The response to ramp depolarization was also studied in 3 old animals (ages 12-19) under Na pentobarbital anesthesia. Of 35 cells studied in these animals, 20 showed S responses, 13 C responses, and 2 MG responses. All populations showed fewer MG responses than were found in previous reports from central neurons in anesthetized or unanesthetized animals. We conclude that the principal cause of damage to the cell membrane is the type of technique used for recording. There is also a tendency for greater proportions of ceiling and minimal gradient responses to be found as a function of age (X^2, $P<.10$) and as a function of pentobarbital anesthesia (X^2, $P<.01$), but, on the basis of the present sampling, the differences relative to age are not sufficiently great to be conclusive, statistically. These results suggest that the plasma membranes of most cells in the motor cortex of older animals are well maintained. Possible artifactual injury to neurons recorded in this way was assessed in separate studies by comparing the response to weak click of intracellularly studied units to that of extracellularly studied units. The responses were comparable, indicating an absence of sufficient injury in penetrated cells to affect their transfer property for weak auditory stimuli. (Supported by AGO 1754).

241.9 LOSS OF PLACE SPECIFICITY IN HIPPOCAMPAL COMPLEX-SPIKE CELLS OF SENESCENT RAT, C. A. Barnes, B. L. McNaughton, and J. O'Keefe*. Cerebral Functions Group, Dept. Anat. & Embryol., University College, London, WC1E 6BT, U.K.

It has previously been shown that granule cells of the rat fascia dentata lose roughly a third of their excitatory input from the perforant pathway between middle and old age. This is partially compensated for, however, by a reduction in the discharge threshold following orthodromic activation and by a strengthening of the remaining excitatory synapses (Barnes and McNaughton, J. Physiol. 309, 473-485, 1980). A possible consequence of this constellation of effects would be that the average firing probability in the granule cell population would remain constant throughout the lifespan, but that cells in the hippocampus of older animals would tend to fire on the basis of less information. Since the granule cells are thought to form the major input stage to the hippocampus, we predicted that the place specificity (O'Keefe and Dostrovski, Brain Res. 34, 171-175, 1971; Olton, Branch, and Best, Exp. Neurol. 58, 387-409, 1978) of hippocampal complex-spike cells (pyramidal cells?) would be disrupted in old animals without a change in the average discharge characteristics of these cells.

The characteristics of "place" cells in the dorsal region of CA1 were recorded from 5 young (10-14 mo) and 5 old (25-29 mo) Fischer-344 rats obtained through the National Institute on Aging. Animals were trained to obtain food reward from the ends of the arms of a radial 8-arm maze. Entry to the arms was controlled by the experimenter so that all 8 arms were visited in a random sequence on each trial. For each cell, 8 such trials were given (64 arm choices total). Single unit activity and the animal's position on the maze were continuously monitored during this behavior by digital computer.

Twenty-seven cells from each age group were studied in this way. No statistically significant differences were found in spike height, spike width, average firing rate, or peak firing rate between age groups. In addition, the mean interspike interval histograms for the two groups were virtually identical. A large and statistically significant difference, however, was found in both the spatial specificity of the firing pattern, and in the reliability of this index from trial to trial (see McNaughton, Barnes, and O'Keefe, 1982 (these abstracts) for a definition of these indices).

If these results are interpreted in terms of the cognitive map theory of hippocampal function, the firing characteristics of the cells of the older rats suggest that these animals should show a behavioral deficit in spatial "perception."

241.10 ULTRASTRUCTURAL STUDIES OF THE AGING FELINE CENTRAL NERVOUS SYSTEM P. J. Leveille* and J. de Vellis. Brain Research Intitute, Univ. of California, Los Angeles, CA 90024.

Correlative microscopic analyses were conducted on routinely prepared ultrathin and semithin sections of selected brain and spinal cord areas from known-aged cats to assess the morphological status of differentiated cell types in the aging feline CNS. The selected areas of interest included: primary auditory and motor cortex, cerebellum, hippocampus and lumbar cord segments. The cats ranged in age from 13 - 19 years and were aldehyde perfused under sodium pentobarbital anesthesia. In general, the findings of this study suggest that the cat - a classical animal model of neurophysiology - will provide a valuable model for comparative gerontological study of age-related changes in neurons, glia, microvasculature and the blood-brain-barrier. The observations supporting this view are summarized below.

Within any given section and from any given region, intracytoplasmic inclusion bodies of lipofuscin "aging" pigment were a notable feature of a number of oligodendrocytic, astrocytic and neuronal profiles as well as the supporting meningeal, endothelial and perithelial elements. Moreover, multiple examples of primary demyelination, Wallerian degeneration, dendritic and axonal vacuolation, and neuronal dyskaryosis and necrosis were observed. Throughout the brain and cord parenchyma, the microvasculature showed a remarkable accumulation of lipid substance which was clearly discernible at the ultrastructural level as well as in toluidine-blue stained semithin sections. Finally, several foci of abnormal neurites were observed in ultrathin sections of the primary auditory cortex. Of particular interest is the finding that these abnormal neurites which are enlarged and are packed with laminated dense bodies and granular, dense mitochondria have the same morphology as the earliest identified precursor of the classical neuritic "senile" plaque - a type of lesion previously found only in human, monkey and dog brain specimens.

241.11 DIETARY RESTRICTION RETARDS AGE-RELATED DECREMENTS IN STEREOTYPY FOLLOWING INTRASTRIATAL INJECTION OF DA-ACTIVE AGENTS. J. A. Joseph, J. Whitaker*, D. K. Ingram*, and G. Roth*. Gerontology Research Center, National Institute on Aging, Baltimore, MD 21224.

Previously, (Levin et al. Science 214, 1981, 561-562) it was shown that dietary restriction (alternate days of feeding and fasting from weaning) in male Wistar rats retards age-associated loss of striatal dopamine (DA) receptor concentrations. Motor behavioral consequences of dietary restriction were examined by assessing differences in rotation, sniffing, and grooming for 10 min following intrastriatal injections (.5µl vols) of amphetamine (AMPH 02.5, 5, 7, 10µg), dopamine (DA, 5, 10, 50µg), atropine (AT, 0, 1, 7µg), or AT-AMPH combinations (1-2.5µg, respectively). Three groups of unilaterally lesioned (6-OHDA, left substantia nigra) animals were used: young ad lib (YAL 6 mo, N=24); old ad lib (N=12, OAL 24 mo); and old restricted (OR, N=9, 24 mo). All animals were screened with 2 mg/kg, AMPH ip., and had to exhibit at least 25 rotations or they were not used. Animals that met criteria then had cannula chronically implanted into the right (intact) striatum. Vehicle for AMPH, AT, was saline; DA vehicle was N_2 bubbled D_2O. Nialamide (50 mg/kg) was given 1.5 hr. prior to DA injections. Each regimen was completed before a new drug was given. Rotations were recorded as a ratio of left over right turns. Results showed that for both AMPH and AT, the YAL and OR animals demonstrated more turns than OAL animals (AMPH $F=(2,42)=6.36$ $p < .004$; AT F $(2,17) = 3.57$ $p < .05$). As the AT doses were increased the YAL and OR animals showed selective increases in rotation while OAL rats did not (age X drug interaction F $(4,34) = 4.67$, $p < .006$). Intrastriatal DA did not produce strong turning in any group. AT-AMPH combinations selectively enhanced rotation only in the YAL group, with OR and OAL groups not differing from each other (age X combination F $(6,51) = 4.53$ $p < .001$). The OR group had a much higher overall frequency of sniffing compared to the OAL group following AMPH (OR 40 $\pm$ 8.6; OAL 19 $\pm$ 5.4; X $\pm$ SEM) or AT (OR 41 $\pm$ 8.4; OAL 23.7 $\pm$ 5.7) and was comparable to YAL for both drugs. DA produced greater sniffing in the OR group than in either YAL or OAL groups (F $(2,27) = 17.26$ $p < .001$; OR 46 $\pm$ 7.7; YAL 14 $\pm$ 3.6; OAL 16 $\pm$ 4.3). Grooming behavior was not significantly affected in any group of animals by any drug. Results suggest dietary restriction which prevents striatal DA-receptor loss during aging may selectively reduce motor behavior decrements seen in senescence-that is, while OR animals responded as YAL animals following AT or AMPH, their responses (turns) were not potentiated following AT-AMPH combinations as in the YAL group. Thus, some motor behavioral decrements remain.

241.12 ETHANOL-INDUCED CHANGES IN BODY TEMPERATURE AND RIGHTING RESPONSE AMONG THREE AGE GROUPS OF MICE. W.G. WOOD and H.J. ARMBRECHT*. VA Medical Ctr., GRECC, and Depts. of Med. and Biochem., St. Louis Univ. Schl. of Med., St. Louis, MO 63125.

Age-related differences have been reported for the effects of acute administration of ethanol. Differences have been observed for measures such as righting response, sleep-time, and motor activity following a single injection of ethanol. Several different mechanisms have been proposed to explain age-related differences in response to ethanol, e.g., metabolic, body water and mass, central nervous system sensitivity. In addition to the above-mentioned explanations, another factor that may contribute to age-related differences is the role of ethanol-induced hypothermia. In several studies it has been suggested that mild hypothermia induced by ethanol may inhibit in part the depressant effects of ethanol. It has been reported that when ethanol-induced hypothermia has been blocked, ethanol depression is increased. Based on these results, it might be predicted that aging animals would differ in their thermoregulatory response to ethanol as compared to younger animals. This experiment examined the effects of ethanol on body temperature and ethanol-induced depression among three different age groups (8 mo, 18 mo, and 28 mo) of C57BL/6NNIA male mice. Animals were injected intraperitoneally with 3 g/kg ethanol or an equivalent volume of saline. Measures included time until the righting response (RR) was lost, duration of the loss, blood ethanol levels when the RR was lost and regained. Rectal temperature was measured prior to injection, when the RR was lost and regained and at 30 and 120 minutes post-injection. The 28 mo group slept significantly longer ($p < .05$) following an ethanol injection as compared to the other groups. The three age groups did not differ significantly when time until RR was lost or blood ethanol levels at loss of RR. When the RR was regained the 28 mo group had significantly lower blood ethanol levels than the 8 mo group ($p < .03$). Changes in ethanol-induced body temperature were related to age. Generally, the old animals showed less temperature change in response to ethanol as compared to younger animals. Significant age differences (p's < .04) occurred at loss and regaining of RR and at 30 and 120 minutes post-injection. Age differences in body temperature prior to ethanol or saline injection were small and nonsignificant. The findings of this study indicate that the ability of aging animals to respond to an ethanol challenge is impaired. In addition, support is provided for the hypothesis that mild hypothermia reduces ethanol depression. The greater ethanol-induced impairment of aging animals as compared to younger animals may result from changes in neuronal membrane fluidity and composition with aging and the effects of ethanol.

242.1 PONTINE LESION SITE DETERMINES THE OCCURRENCE OF ATTACK BEHAVIOR IN CATS DURING PARADOXICAL SLEEP WITHOUT ATONIA AND WAKEFULNESS. C.E. Washington and A.R. Morrison, Sch. of Vet. Med., Univ. of Pa., Phila., Pa.,19104

Cats with small, bilateral, dorsolateral pontine tegmental lesions no longer have atonia during paradoxical sleep (PS). Larger lesions that extend rostroventrally into the caudal midbrain tegmentum lead to dramatic behavior resembling attack during "PS without atonia". An increase in aggressiveness was also observed during wakefulness (W) in some cats (Hendricks et al., Brain Res., 1982, In press). To what extent is attack behavior in PS related to increased aggressiveness in W ?

Fourteen cats were lesioned electrolytically. Three exhibited attack behavior during PS and W and 1 during PS only. This developed post-operatively between 8-26 days in PS and 2-26 days in W. The different syndromes observed varied in relation to lesion site. Lesions extending rostroventrally into the midbrain tegmentum at P=2.5, H=2.5, V=-4.0 (Snider and Niemer) resulted in attack during PS and W, while 1 lesion centered ventrally in the pons at P=3.0, H=2.0, V=-5.0 but without a rostral extension induced attack behavior during PS only. Behaviors during W were different from those occurring during PS. Well coordinated, affective attacks against conspecifics and experimenter occurred during systematic testing. Although there was no increase in predation in W, attacks during PS were more predatory in nature. Only 2 of 10 cats with lesions located at P=3.0, H= 2.0 or 2.5, and V=-3.0 or -4.0 and no attack during PS without atonia were aggressive in W.

Sastre and Jouvet (Physiol. Behav., 22; 979, 1979) suggest on the basis of large lesions that elaborate behavior in PS, including aggressive-like behavior, depends only on destruction of neurons producing atonia. Our results demonstrate that additional systems must be involved and that these course through the tegmentum, presumably extending from limbic structures (Washington, et al., Sleep Res.,10 1982). Furthermore, environmental influences in W altered the lesion effects in a qualitative way, reflecting the overlapping of systems controlling elements of agonistic behaviors (Leyhausen, Cat Behavior, 1979). (Supported by NS 13110, MH 15092 and the Harry Frank Guggenheim Foundation)

242.2 RATE DIFFERENCES OF REM SLEEP OFF CELLS IN BRAINSTEM NUCLEI OF THE CAT. J. P. Nelson, R. W. McCarley and J. A. Hobson Laboratory of Neurophysiology, Dept. of Psychiatry, Harvard Medical School, Boston, MA 02115.

We have recorded 55 cells which decrease their mean firing rate during REM sleep(D) by at least 50% compared with waking(W) and synchronized sleep(S). Each cell was recorded during at least one complete sleep cycle. These cells are drawn from a population of over 2000 cells recorded in the brainstem of 25 cats over an area extending from the rostral medulla to the rostral midbrain, and laterally to 4.0 mm.

Of these D-off cells 71% are found in only three relatively small nuclear groups, all known to contain many aminergic cell bodies: two raphe nuclei (N=18 of 25), the locus coeruleus (N=16 of 38), and the peribrachial nuclei (N=5 of 15). 10 others were uncommon members of a population of 279 reticular neurons. The other six D-off cells were scattered. Taken together these groups comprise a plate of cells which extends from the raphe across the dorsal tegmentum at the ponto-medullary junction and down along the brachium conjunctivum as it courses toward the red nucleus. The reticular group of off-cells lies medial to the brachium conjuctivum in the central tegmental field and might be strays from the larger locus coeruleus/peribrachial group.

	raphe	coerul.	peribr.	retic
N:	18	16	5	10
geometric means				
W	2.14	3.63	3.46	9.33
S	1.02	2.00	2.40	5.62
D	0.03	0.10	0.05	1.91
selectivity ratios				
W/D	75.9	36.3	74.1	4.9
S/D	34.0	20.0	48.0	2.9

Rate data show, however, that the reticular group differs from the other 3 nuclear groups. The reticular cells fire significantly faster in all states than the other groups(see table). Also, the selectivity of the reticular cells in W was much lower than in the other groups, that is they decreased rates much less passing from W to D. These differences were significant at 0.02 level or better for all the groups.

This research was supported by NIMH grant 2 R01 MH 13923-15, and a RSDA MH00280 to RWM.

242.3 CEREBRAL POTENTIALS PRECEDING THE RAPID EYE MOVEMENTS OF REM SLEEP: SIMILARITIES WITH PGO WAVES AND WAKING POTENTIALS. J. W. Winkelman*, F. H. Duffy and R. W. McCarley (SPON: G. Cassens). Depts. of Psychiatry and Neurology, Harvard Medical School, Boston, MA 02115

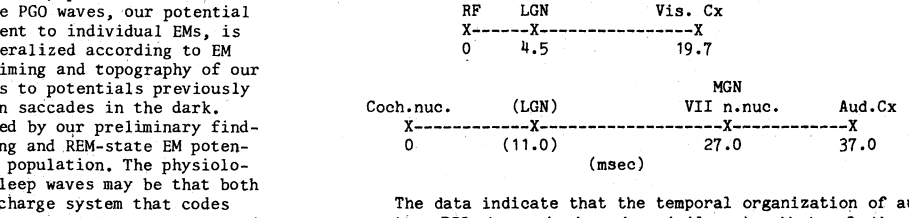
PGO waves in animals are one of the defining characteristics of REM sleep, occurring in temporal association with individual rapid eye movements, and lateralized according to direction of eye movement. However, they have not been detected in humans. Using the onset of individual eye movements in each lateral direction (N>100) of REM sleep as a time zero for triggering, averaged event related potentials were produced: these extended from .25 sec before to .25 sec after eye movement (EM) onset in 5 subjects. These averages were then displayed in 4 msec epochs as color representations of the voltages of 4096 points derived from the standard 10-20 electrode array by linear interpolation (BEAM system). A sharp positive potential was found which immediately preceded EM onset, and had a focus of maximal voltage in the temporo-occipital region contralateral to EM direction. The complete waveform consisted of an early positive ramp (onset mean = -27.4 msec) leading into the sharp positive spike (onset = -7.6 msec; duration = 22.8 msec), followed immediately by a low voltage, longer duration negative wave. These cerebral REM potentials could be clearly distinguished from the more anterior and subsequent corneo-retinal potential.

By its striking resemblance to two neuroelectric events previously considered disparate - PGO waves in animals and waking EM potentials in man - this REM potential may provide the link between these two objects of study. Like PGO waves, our potential occurs in REM sleep immediately antecedent to individual EMs, is in posterior cortical areas, and is lateralized according to EM direction. In addition, the waveform, timing and topography of our REM potential nearly exactly corresponds to potentials previously found to be produced during waking human saccades in the dark. This latter conclusion has been supported by our preliminary finding of a close resemblance between waking and REM-state EM potentials within individual subjects of our population. The physiological link between the waking and REM sleep waves may be that both represent activation of a corollary discharge system that codes for parameters of eye movement.

242.4 TEMPORAL ORGANIZATION OF PGO WAVE TRANSMISSION DURING REM SLEEP IN THE AUDITORY AND VISUAL SYSTEMS IN THE CAT. H.P. Roffwarg, P. Gatz*, J. Farber. Department of Psychiatry, University of Texas Health Science Center, Dallas, Texas 75235.

We have previously reported that the auditory system in cat and man is phasically activated during REM sleep in a pattern similar to the activation of the visual system in REM sleep (Pessah and Roffwarg, Science, 178: 773, 1972; Farber et al, APSS, 1979). In addition to middle muscle activity (MEMA) being present in REM sleep, we have recorded in the VII n. nuc., the cochlear nucleus and the auditory cortex PGO-type waves associated with REM sleep. The morphology and distribution of these waves are similar to PGO's recorded in the visual system. This study was designed to identify the temporal organization of auditory system PGO's and their temporal relationship to visual system PGO's.

Cats with electrodes chronically implanted in visual (LGN, visual cortex) and auditory (cochlear nucleus, MGN, auditory cortex, VII n. nuc.) systems, and reticular formation (RF) were polygraphically recorded during numerous sleep-wake cycles. From magnetic tape recordings, auditory or visual system PGO's were used to generate averaged transient events from the other polygraphically recorded sites. Averaged events were summated over at least 30 PGO waves. Time intervals between the averaged events and triggering PGO were calculated.



The data indicate that the temporal organization of auditory system PGO transmission is similar to that of the visual system. From data on auditory system evoked reponses to sound stimulation we find that the sequential pattern in REM sleep is also similar to that of the awake state.

242.5 ALTERATION OF PARADOXICAL SLEEP FOLLOWING INTERRUPTION OF RETICULAR PATHWAYS AT THE PONTOMEDULLARY JUNCTION. H. Webster, L. Friedman and B.E. Jones. Lab. of Neuroanatomy, Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4.

Previous studies, employing complete transection of the brain stem at levels between the midbrain and the medulla have demonstrated that the pontine reticular formation may generate PGO spikes and neck muscle atonia, which respectively correspond to the cerebral and peripheral manifestations of paradoxical sleep. In order to investigate further the role of the pontine reticular formation and its efferent and afferent connections in the generation of these parameters, bilateral transverse sections were made through the tegmentum at the pontomedullary junction with the aid of a retractable tungsten wire knife.

Daily polygraphic records were obtained from chronically implanted cats for 1 week before and 3 weeks after the knife cut. Electrographic parameters, including EEG, PGO, EMG, EOG and OBS (Olfactory Bulb Spindles) were also quantified, stored and analyzed by computer graphics and cluster analysis as described previously (Friedman and Jones, Neurosci. Abst. 7: 233, 1981). The extent of the transection was examined in Kluver-Berrera stained sagittal sections. In 7 cats, the cuts passed caudal to the 6 n.nuc. dorsally and rostral to the 7 n.nuc. ventrally and traversed the entire medial and lateral tegmentum. The dorsal-ventral extension of the cut varied to include to a differing extent the dorsal or ventral pathways within the tegmentum. However, the peripherally located ventral and lateral long ascending and descending tracts were spared as well as the structures dorsal to the tegmentum.

The most dorsal transection, which cut through the region where catecholamine fibers descend from the locus coeruleus, did not alter the ascending or descending manifestations of paradoxical sleep. Conversely, the most ventrally placed cut, which did not include the latter region, produced a loss of muscle atonia while preserving periods of paradoxical sleep defined by PGO spikes and REM in association with an activated cortex and in absence of olfactory bulb spindles. A greater disruption of paradoxical sleep characterized by shortened episodes and a reduction of PGO spike rate in addition to the loss of atonia was observed following more centrally placed cuts which interrupted the majority of reticular connections.

These results indicate that the pontine reticular formation is essential for muscle atonia and may be sufficient for the occurence of PGO spikes in association with an activated cortex. However, the integrity of the pontomedullary reticular formation is necessary for the full coordination of the defining variables of paradoxical sleep. (Supported by MA 6464, Medical Research Council of Canada).

242.6 DEOXYCOFORMYCIN, AN ADENOSINE DEAMINASE INHIBITOR, INCREASES SLEEP IN RATS. M. Radulovacki, R.M. Virus*, M. Djuricic-Nedelson* and R.D. Green* (SPON: J. Javaid). Dept. of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60680.

Preliminary data reported by Haulica et al. (1) indicate a possible hypnogenic role for adenosine. The present work tested the hypothesis that increase of endogenous adenosine produced by administration of deoxycoformycin, an inhibitor of adenosine deaminase, may promote sleep in rats.

Rats were implanted for EEG and EMG recording. After receiving either saline (i.p.) or deoxycoformycin (0.5 mg/kg, i.p.), animals were continuously recorded for 6 h. Their EEG records were analyzed as wakefulness, slow-wave sleep (S_1 and S_2) and REM sleep.

Condition	Hours	Saline	Deoxycoformycin
W	0 - 3	40 ± 5	28 ± 3*
	0 - 6	85 ± 9	57 ± 5*
S_1	0 - 3	49 ± 2	35 ± 5*
	0 - 6	96 ± 5	72 ± 8*
S_2	0 - 3	76 ± 6	93 ± 4*
	0 - 6	144 ±13	178 ±10
REM	0 - 3	14 ± 2	23 ± 1*
	0 - 6	33 ± 2	49 ± 1**
S_2 lat.(min)		14 ± 4	11 ± 2
REMS lat.(min)		55 ± 6	22 ± 4**

All results expressed as means ± S.E.M. (min) of 6 rats.
*p <0.05, **p< 0.01 by Studentized range test.

The results clearly show that administration of deoxycoformycin to rats increased S_2 and REM sleep, decreased wakefulness and S_1 and reduced REM sleep latency by over 50%. These findings indicate hypnogenic role for endogenous adenosine and are consistent with the reports on the effects of metabolically stable adenosine analogs on sleep (2,3). (Supported by ONR contract N000 14-79-C-0420).

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242.7 ENHANCEMENT OF SLOW WAVE SLEEP (S_2) AND SUPPRESSION OF REM SLEEP BY A STABLE ADENOSINE ANALOG, L-N^6-PHENYL-ISOPROPYLADENOSINE R.M. Virus*, M. Djuricic-Nedelson*, M. Radulovacki and R.D. Green.* Department of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60680.

Recent experimental evidence suggests that adenosine is a neurotransmitter or neuromodulator in the CNS (1) and may be involved in the regulation of sleep and wakefulness (2,3). The present experiments examined the effectsof an adenosine receptor agonist, L-N^6-phenylisopropyladenosine (L-PIA), on the sleep-wakefulness cycle.

EEG and EMG recordings were performed in freely moving adult male rats and the behavioral state of the animals was classified as wakefulness (W), slow wave sleep 1 (S_1), slow wave sleep 2 (S_2), or rapid eye movement sleep (REM). All rats received i.p. injections of either one of 3 doses of L-PIA or 0.9% saline vehicle in a volume of 1 ml/kg as shown in the table below:

Behavioral State	L-PIA DOSE IN mg/kg (μmoles/kg) 0 (Saline)	0.04 (0.1)	0.12 (0.3)	0.36 (0.9)
W	49.8 ± 10.9	50.5 ± 8.4	42.2 ± 5.8	74.0 ± 7.8
S_1	44.8 ± 4.1	24.8 ± 3.8	25.0 ± 1.4	33.8 ± 6.2
S_2	68.8 ± 9.5	90.2 ± 8.1	98.8 ± 2.3*	71.8 ± 7.5
REM	14.0 ± 2.0	14.5 ± 3.5	14.0 ± 3.4	0.5 ± 0.5*

All values reported are means ± S.E.M. in minutes during the first three hours of recording. Each group consisted of 4 animals.
*Significantly different from saline control groups (P<0.01).

These data demonstrate that 0.12 mg/kg of L-PIA significantly increased the duration of S_2 (+44%, P<0.01) and that the 0.36 mg/kg dose produced a highly significant suppression of REM sleep (-96%, P<<0.01). The increase in S_2 produced by 0.12 mg/kg of L-PIA was consistent with earlier reports of sedative and hypnotic actions of adenosine (2) and its analogs (3,4). The nearly complete suppression of REM sleep produced by 0.35 mg/kg of L-PIA may involve reduced release of neurotransmitters or an enhanced accumulation of cAMP after adenosine receptor stimulation (1). (Supported by ONR Contract N00014-79-C-0420).

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242.8 MURAMYL DIPEPTIDE INDUCES NON-REM SLEEP IN SQUIRREL MONKEYS. D.B. Wexler, C.J. Harling* and M.C. Moore-Ede. Dept. of Physiology & Biophysics, Harvard Medical School, Boston MA 02115.

The recently purified endogenous sleep-promoting factor "S" is a low molecular weight glycopeptide containing muramic acid (J. Biol. Chem. 257: 1664, 1982). A synthetic muramyl dipeptide (MDP: N-Ac-Muramyl-L-Alanyl-D-Isoglutamine) induces slow-wave sleep in rabbits and cats (J.R. Pappenheimer, personal communication). We are examining whether MDP influences the circadian oscillator which times the sleep-wake cycle in the diurnal squirrel monkey (*Saimiri sciureus*). Two monkeys (~900gm) were prepared with an acrylic headcap and swivel commutator for chronic recording of EEG, EOG, EMG and core body temperature in free-ranging conditions and separately housed in constant dim illumination (60 lux) for at least 1 week to establish a free-running sleep-wake rhythm. On the experimental day, a single dose of MDP (50 nanomoles in 1.25cc saline) was given by IV infusion or IP injection during early subjective day, 1 hr after polygraphically-determined wakeup time. Polygraphic records were made (paper speed 1.5mm/sec) during baseline, experimental and follow-up days, scored in 1-min epochs. Percentage time spent in each state on experimental and follow-up days were compared with baseline data, matched for circadian time of day (i.e., subjective day and subjective night). After MDP was injected, episodes of NREM sleep occurred throughout subjective day, occurring 65% of the time, compared to baseline values of <10% for the 1st monkey and 40% for the 2nd monkey. REM sleep remained at 0% of the subjective day after MDP despite the increase in NREM sleep. Changes between waking and NREM sleep states were similar to those seen in baseline subjective nights. Following the induced daytime sleep, monkeys exhibited sleep episodes during the circadian night which were indistinguishable from those recorded on baseline subjective nights: awake 20-40%; NREM 50-70%; REM ~10%. The sleep-wake pattern was normal on the day after MDP was given and had the circadian sleep onset and wake times predicted by the prior free-running period. MDP also produced a 1 to 1.5°C elevation in body temperature which lasted through the subjective day. These preliminary results indicate that MDP given early in the subjective day induces non-REM but not REM sleep in the squirrel monkey. Timing of circadian temperature and sleep-wake cycles does not appear to be altered on the subsequent day. Further studies using MDP at other times of day, with more detailed analysis are needed to assess the effects of this agent on the physiological processes of sleep. (NS13921;AFOSR78-3560.)

242.9 ALTERATION OF SLEEP RELATED NEURONAL ACTIVITY AFTER LOCAL SCOPOLAMINE INFUSION. G.A. Marks, S.G. Speciale, A. Foote*, H.P. Roffwarg. Department of Psychiatry, University of Texas Health Science Center, Dallas, Texas 75235.

Activity of single cells in widespread brain areas is influenced differentially by sleep stage. The brain mechanisms controlling sleep stage specific cellular activity is unknown, but due to its ubiquitous appearance, it is likely to be controlled by a few widely projecting systems. We are using the dorsal lateral geniculate nucleus (dLGN) of the rat as a model to study sleep-related influences on cell firing. Over 90% of its cells (P-cells) exhibit sleep stage related activity and discharge rates are in excess of 2:1 in REM sleep vs. slow wave sleep (SWS). Many of the dLGN afferents are identified and characterized, and sleep-related alterations in activity appear to be independent of the dLGN's unique role in the visual system since these relative changes are maintained after bilateral enucleation.

Our strategy is to perform functional deafferentations of specific dLGN inputs while monitoring sleep-related activity pre- and post-drug infusion with activity in the contralateral nucleus serving as a control. We have developed a technique to inject pharmacological agents intracerebrally into cell populations from which we are monitoring multiple unit activity. Serotonin depleting doses of 5,7-DHT did not have a differential effect across sleep stages, although activity increased relative to control. Norepinephrine depleting doses of 6-OHDA decreased activity in all stages, however, differential decreases in rate in SWS and in the transition to REM sleep were observed. The muscarinic receptor blocking agent scopolamine (0.2ul of 10 mg/ml scopolamine HCl pressure injected into the dLGN) produces a reversible general reduction in activity in every stage. The rates of activity are differentially reduced in REM sleep resulting in significant reductions in the REM/SWS ratio; increases in the AW/REM ratio; and no significant changes in the AW/SWS ratio. The characteristic increases in mean discharge rate in SWS at the transition to REM sleep is still observed after drug administration, even in the instances where REM sleep rates fall below that of the preceeding SWS episode.

These preliminary data suggest that the high mean discharge rates observed in REM sleep in the dLGN is under the influence of a muscarinic cholinergic mechanism while the increased rates observed in the transition from SWS to REM sleep is under the influence of a noradrenergic mechanism.

This work has been partially supported by NIH grants: MH3630 EY03327, MH31402.

242.10 ACUTE AND CHRONIC EFFECTS OF PARA-CHLOROAMPHETAMINE (PCA) ON SLEEP AND RESPIRATORY RATE IN THE RAT. S. De Mesquita. Dept. of Physiol. Marshall Univ. Sch. of Med., Huntington, WV 25701

Acutely PCA causes release of endogenous brain serotonin (5-HT) while its long term effects are associated with brain 5-HT depletion. Alterations in sleep and respiratory rate during sleep were related to acute and chronic changes in 5-HT metabolism.

Six male SD rats with chronic electrodes were monitored during sleep for three successive days both before and after PCA treatment (2mg/kg ip). Respiratory rate was obtained by thoracic pneumography. A mean control value (C) was calculated for each sleep and respiratory parameter and compared with the means from each of the experimental runs (E1, E2, E3) occurring 0, 24 and 48 hrs after the PCA treatment.

In addition to increasing NR (Non-Rapid Eye Movement) and R (Rapid Eye Movement) sleep latencies, the acute effect of PCA treatment caused a decrease in TST% (total sleep time/total run time), REM% (total R time/total sleep time) and the no. of R periods (REMPS)/hr of NR sleep.

	C (mean ± SD)	E1	
NR latency (min)	25.9± 9.5	372.7±103.8	**
R latency (min after NR onset)	21.5± 9.1	165.5± 90.9	*
TST%	69.0±13.1	31.7± 15.1	**
REM%	18.0± 4.6	4.7± 3.9	**
# REMPS/hr NR	5.1± 1.3	0.9± 0.8	**

TST% and REM% in E2 and E3 were not changed from C, both R and NR prior to R, durations were siginifcantly shorter in E3 than E1; only NR duration (5.8±1.1 min in E3) was significantly ($P<0.05$) shorter than C. The number of REMPS/hr of NR sleep progressively increased from E1 to E2 and E3, however the rate in E3 (6.6±1.4) was not different from C.

PCA acutely depressed the respiratory rate in both NR and R, while NR respiratory rate returned to normal within 24 hrs, R respiratory rate remained depressed.

	Respiratory Rate (breaths/min)			
	C	E1	E2	E3
NR	111.6± 4.3	106.9± 6.1*	111.7±3.0	110.0±5.0
R	132.0±18.3	107.3±12.8**	121.3±5.9*	121.5±5.1*

Acute release of 5-HT increased sleep latency, decreased TST%, REM% and respiratory rate in NR and R. Partial depletion of brain 5-HT was associated with normal TST%, REM%, however there tended to be an acceleration of the NR/R phase switching, such that NR periods prior to R became shorter and REMPS more frequent. Chronically PCA effected sleep respiratory rate differentially. NR respiratory rate returned to normal, while R respiratory rate remained significantly lower than C.

** sign diff from C, $P<0.01$; * sign diff from C, $P<0.05$.

242.11 EFFECTS OF SEROTONERGIC RECEPTOR BLOCKADE ON SLEEP IN RATS. C. Fornal* and M. Radulovacki (SPON: J. Hughes). Department of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60680.

It has been suggested that reduced activity of central serotonergic neurons may be necessary for the onset of REM sleep (REMS). This hypothesis is supported by the almost complete suppression of dorsal raphe neuronal discharge and release of 5-HT at cortical nerve terminals during REMS.

The present study investigated the effects of decreased serotonergic activity on sleep by examining the effects of metergoline, a highly selective 5-HT receptor antagonist. As a measure of the degree and duration of blockade of central serotonergic receptors, we also examined the ability of metergoline to antagonize the head-shakes induced by quipazine, a direct 5-HT receptor agonist. Previous studies have demonstrated that head-shaking in rodents is a useful model for increased serotonergic activity.

Male Sprague-Dawley rats implanted with EEG and EMG electrodes received i.p. injections of metergoline (1.0, 2.5, and 5.0 mg/kg) or drug vehicle and were polygraphically recorded for 12 hrs. All records were analyzed for wakefulness (W), slow-wave sleep (SWS), and REMS. Another group of rats similarly treated with metergoline received i.p. injections of quipazine (5 mg/kg) at 1, 6, and 12 hrs after metergoline pretreatment. The number of head-shakes occurring over the next hr after quipazine administration was determined.

Results indicate that REMS was significantly reduced by 48 and 52% respectively during the first 6 hrs after administration of 2.5 and 5.0 mg/kg of metergoline, as compared to control. SWS was also reduced and W was increased by 5.0 mg/kg of metergoline. One mg/kg of metergoline had no effect on sleep and wakefulness but completely prevented quipazine-induced head-shakes in rats at 1 and 6 hrs after drug administration.

These data do not support the hypothesis that reduced central serotonergic activity promotes REMS in rats. (Supported by ONR Contract N00014-79-C-0420).

242.12 CHANGES IN NE AND 5HT METABOLISM IN DISCRETE BRAIN AREAS FOLLOWING REM SLEEP DEPRIVATION IN RATS. L.A. Mattiace, C. Johnston, A. Negro-Vilar, J. Farber (SPON: J. Herman). Departments of Physiology and Psychiatry, University of Texas Health Science Center, Dallas, Texas 75235.

REM sleep deprivation affects the monoaminergic system differentially. Striatal dopamine (DA) turnover is increased when measured immediately following REM sleep deprivation (Farber et al, Neurosci.Abst., 8, 1982) and during REM sleep rebound (Wojcik and Radulovacki, Neurosci.Abst., 6: 52, 1980), whereas forebrain norepinephrine (NE) and serotonin (5HT) metabolite levels in REM sleep deprived rats were not different from levels in stress-control animals. In this study we attempted to delineate specific brain structures where 5HT and NE metabolism may be affected by REM sleep deprivation. Using the pedestal-water technique of REM sleep deprivation, male rats (370-400gms) were kept for 4 days on an 8cm platform (Rd, N=4), or on a 14cm stress-control platform (SC, N=4), or in their home cages (C, N=4). The rats were sacrificed immediately after removal from the pedestals. The brain areas, dissected by micropunch procedure, included the frontal cortex (FC), striatum (ST), midbrain ventral tegmental area (VTA) and the medial (MR) and dorsal raphe (DR) nuclei.

5HT and NE and their respective metabolites, 5HIAA and MHPG, were analyzed using high performance liquid chromatography with electrochemical detection.

		NE	MHPG	5HT	5HIAA
	Rd	9.9 ±1.0	6.5 ±0.7*	11.9 ±1.1*	3.03 ±.31*
FC	SC	9.6 ±0.9	2.3 ±0.3	8.0 ±0.7	2.61 ±.30
	C	10.1 ±1.1	2.4 ±0.2	7.4 ±0.8	1.88 ±.21
	Rd	1.34 ±0.14	0.96 ±.08*	14.5 ±1.0	7.16 ±.81*
ST	SC	1.50 ±0.14	0.23 ±.02*	13.8 ±1.3	6.52 ±.70
	C	1.96 ±0.21	0.47 ±.05	13.0 ±1.3	4.72 ±.52
	Rd	9.50 ±.91*	2.08 ±.23*	10.6 ±1.1*	2.14 ±.23*
VTA	SC	6.83 ±.66	1.02 ±.10	7.3 ±0.6	1.23 ±.11
	C	6.83 ±.70	1.47 ±.13	6.9 ±0.6	1.33 ±.13
	Rd	4.35 ±.44	0.60 ±.05*	22.7 ±2.4*	8.32 ± 0.82*
DR	SC	5.30 ±.51	0.29 ±.03	28.3 ±2.7*	8.20 ± 0.80*
	C	4.53 ±.50	0.43 ±.05	18.8 ±1.9	6.03 ± 0.58

*$p < 0.05$, means ± SEM (ng/mg protein)

The data show that 5HT metabolism increases following REM deprivation in the areas of the FC, VTA, and ST, whereas NE metabolism increases as a result of REM deprivation in the FC, VTA, ST, and DR. 5HT and NE metabolism in the MR remain unaffected across conditions.

242.13 1-PROPRANOLOL, THE β-ADRENERGIC RECEPTOR BLOCKER, HAS NO EFFECTS ON SLEEP IN RATS. R.C. Walovitch and M. Radulovacki. Dept. of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612

Previous results in our laboratory indicate that administration of α-adrenergic receptor blockers selectively decreases REM sleep (REMS) in rats (1,2). The present study determined the effects on sleep in rats of the β-adrenoreceptor blocker, l-propranolol. D-propranolol which possesses no appreciable affinity for β-adrenoreceptor but shares other properties with l-propranolol was also administered.

Three month old, male, Sprague-Dawley rats (280-310g) were implanted with EEG and EMG electrodes, and a week following surgery, animals were given either d or l-propranolol (10 mg/kg i.p.). Immediately following administration of drugs, animals were polygraphically recorded for 6 hr from 10.00 to 16.00. All polygraphic recordings were analyzed as wakefulness (W) slow wave sleep 1 or 2 (S_1 or S_2) or REMS.

Table 1	W	S_1	S_2	REM
Vehicle (propylene glycol)	81 ± 9	68 ± 4	173 ± 10	36 ± 2
d-Propranolol	77 ± 10	61 ± 11	188 ± 10	34 ± 4
l-Propranolol	86 ± 17	66 ± 7	186 ± 18	22 ± 6

The results are mean ± S.E. min for 6 animals. Statistical analysis was done by one-way ANOVA with multiple comparisons of l-Propranolol to either d-propranolol or vehicle by Scheffe' test.

From table 1, it can be seen that during the 6 hr recording period neither l or d-propranolol has any significant effect on the sleep-wake cycle of rats. Although it has been reported that dl-propranolol produced a short term suppression of REMS in rats (3), statistical analysis of the first 3 hr as well as first 4 hr of our polygraphic recording failed to support that finding. Present results indicate that in contrast to the effects of α-adrenoreceptor blockade of REMS, blockade of β-adrenoreceptor did not produce a significant suppression of REMS. (Supported by ONR Contract N00014-79-C-0420).

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242.14 NONCORRESPONDENCE OF RAPID EYE MOVEMENT SLEEP DEPRIVATION AND CORTICAL β-ADRENERGIC RECEPTOR REGULATION. F. Villegas*, S. Steiner, J. Abreu*, F. Gimino*, B. Beer, M. Abel* and L. Meyerson. Dept. of Psychology, City Univ. of New York, New York, NY 10031 and Dept. of CNS Research, Medical Research Division of American Cyanamid Co., Lederle Laboratories, Pearl River, NY 10965.

Rapid eye movement sleep deprivation (REMD) has been reported to be comparably efficacious to imipramine in the treatment of endogenous depression (Vogel *et al*, 1975). Several other regimens of therapy (i.e. ECT and pharmacotherapy) are known to produce a decrease in the density of β-adrenergic receptors in rodents. REMD has been shown to produce either a reduction (Mogilnicka *et al*, 1980) or no effect (Radulovacki and Micovic, 1982) on rat cortical β-adrenergic receptor density. In an attempt to clarify these discrepancies regarding the relationship between REMD and the β-adrenergic system, the following studies were undertaken. Male Wistar rats were subjected to 72 hours of REMD by the "flower pot" method according to the procedure of Ellman *et al*,(1978). Experimental groups consisted of appropriate cage controls and 72 hour REMD animals on either 6, 9 or 12 cm platforms. The effect of 24 hour recovery from 72 hour REMD on the β-adrenergic system was also studied. Furthermore, EEG recordings were obtained to verify and quantify the degree of REMD, slow-wave sleep and total sleep. β-adrenergic receptor density (Bmax) and affinity constants (K_d) were determined by Scatchard analysis of saturation isotherms employing ^{3}H-dihydroalprenolol (DHA) binding to membranes dissected from frontal cortex. Control values for Bmax and K_d were 6.01 ± 0.39 pmoles/g tissue and 0.28 ± 0.06 nM, respectively. Although the amount of time animals spent in REM was decreased 93% in the 6 and 9 cm platform groups and slow-wave sleep decreased 45%, there were no significant differences in any experimental group in either Bmax or K_d for ^{3}H-DHA binding parameters. Preliminary observations suggest that environmental stress alone as well as combined sleep deprivation and stress appear to diminish the density of β-adrenergic receptors in rat cortical tissues. The results of this study are in agreement with those of Radulovacki and Micovic which state that REMD has no effect on the cortical rodent β-adrenergic receptor. Although REMD may have utility in treating the depressive syndrome in human subjects, naive rodents may not be an adequate model to characterize this phenomenon. Alternatively, the β-adrenergic recognition site in humans maybe more subject to modulation than the rodent receptor. The notion that the antidepressive effect of REMD in humans is operant through the β-adrenergic receptor system still remains nebulous.

242.15 PHENOXYBENZAMINE INHIBITS THE INCREASE IN REM SLEEP OBSERVED IN RATS UNDERGOING ETHANOL WITHDRAWAL. N. Micovic,* S. Culp,* M. Radulovacki and B. Tabakoff. Alcohol and Drug Abuse Research and Training Program., Departments of Pharmacology and Physiology & Biophysics, University of Illinois at the Medical Center, Chicago, IL 60612.

Administration of phenoxybenzamine (PBZ), an α-noradrenergic receptor antagonist, was shown to prevent the increase in REM sleep (REMS) observed in rats after REMS deprivation [Radulovacki et al., Pharmacol. Biochem. Behav., 13: 51-55, 1980). These findings suggested that decreased noradrenergic activity may inhibit REMS. Since an increase in REMS is also observed during ethanol (EtOH) withdrawal [Mendelson et al., J. Stud. Alcohol, 39: 1213-1223, 1978], the present study examined the effects of PBZ on sleep and wakefulness in rats undergoing EtOH withdrawal.

Male Sprague-Dawley rats implanted with EEG and EMG electrodes were placed in ethanol inhalation chambers and exposed to EtOH vapors for seven days. The concentration of EtOH in the chamber air was regulated to maintain blood EtOH levels between 150-300 mg% in the animals. Another group of implanted rats was maintained in similar chambers, though not exposed to EtOH. After the seven-day treatment with ethanol, the animals were removed from the inhalation chambers and were polygraphically recorded for four (4) consecutive days; control rats were treated in a similar manner. On the third day of recording, EtOH-treated rats were subdivided into two groups. One group received an ip injection of PBZ (10 mg/kg), the other the drug vehicle. Records were analyzed for wakefulness (W), slow-wave sleep 1 and 2 (S_1 and S_2), and REMS. The behavior of the animals was monitored for alcohol withdrawal symptoms throughout the four-day recording period.

Overt signs of alcohol withdrawal (piloerection, tremors and seizures) were evident, primarily during the first 24 hours after removal of the animals from the chambers. Electrophysiologic records demonstrated that REMS and S_2 were markedly reduced during the first 24 hours after EtOH withdrawal, while W was increased. Near normal sleep patterns were observed during the second 24-hour period. A significant increase in REMS was seen in EtOH-treated rats 48-96 hours after EtOH withdrawal. This increase in REMS was totally prevented in rats treated with PBZ. The decrease in REMS produced by PBZ was accompanied by a significant increase in S_2 and decrease in W.

These data show that PBZ prevents the increase in REMS observed in rats undergoing EtOH withdrawal. Since withdrawal symptoms are associated with increases in REMS, PBZ may be useful in the treatment of alcohol withdrawal.

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242.16 DIFFERENTIAL EFFECTS OF REM SLEEP DEPRIVATION ON DOPAMINE METABOLISM AND RECEPTOR SENSITIVITY IN STRIATUM AND FRONTAL CORTEX IN THE RAT. J. Farber, J.D. Miller, K.A. Crawford*, B.A. McMillen. Departments of Psychiatry and Pharmacology, University of Texas Health Science Center, Dallas, Texas 75235.

Pronounced behavioral supersensitivity to directly- and indirectly-acting dopamine agonists is known to occur following prolonged REM sleep deprivation. This study was designed to investigate possible changes in dopamine (DA) metabolism, and pre-and post-synaptic receptor sensitivity in the nigro striatal and mesocortical dopaminergic projection systems.

Using the "flower-pot" technique, male rats (Sprague Dawley, 240-270g), were REM sleep deprived for 96 hours on small platforms (6.7cm) surrounded by water. Stress-control rats were placed on 12.8cm platforms. Another group of normal control rats were kept in their home cages under the same light-dark conditions as the other two groups. The rats were sacrificed immediately after removal from the pedestals. DOPAC concentration was measured in striatum and frontal cortex. Scatchard analysis of spiperone binding was used to determine B_{max} and K_d for striatal DA_2 receptors. Displacement of spiperone by mianserine or mianserine plus d-butaclamol was used to determine $5HT_2$ and DA_2 binding in frontal cortex. In addition, apomorphine inhibition of synaptosomal tyrosine hydroxylase was used to determine changes in autoreceptor sensitivity.

Four days of REM sleep deprivation induced a highly significant ($p<.01$) elevation in striatal DOPAC (2.06±.11ug/g) relative to normal controls (1.40±.15). The stress control group exhibited no significant change in striatal DOPAC levels. No significant changes in frontal cortex DOPAC levels were observed in either the REM sleep deprived or stress control groups. No changes in spiperone binding (DA_2 or $5HT_2$ receptors) occurred in the striatum or in the frontal cortex in the various groups. No change in striatal autoreceptor sensitivity was observed in the REM deprivation group: 56% vs. 52% (in normal control) inhibition of tyrosine hydroxylase activity by 0.5uM apomorphine.

The data indicate that behavioral supersensitivity following REM sleep deprivation is not due to altered pre- or post-synaptic receptor sensitivity. The elevation in striatal DOPAC concentration could represent the sequelae of a REM deprivation induced increase in the incidence of spontaneously active DA neurons in the SN. The antidepressant action in humans of MAO inhibitors, ECS and REM deprivation may reflect a similar underlying mechanism.

242.17 CHOLINERGIC STIMULATION OF PONTINE CELLS BY MINI-PUMP INFUSION AUGMENTS PARADOXICAL SLEEP FOR FOURTEEN DAYS. P. Shiromani, J. Barnett* and W. Fishbein. Psychobiology Laboratory, Dept., of Psychology, The City College, CUNY, New York, N.Y. 10031

Converging evidence from a number of studies indicates that a cholinergic system originating in the pontine reticular formation (PRF) may be responsible for generating cortical desynchronization and theta activity during paradoxical sleep (PS). While data exist to indicate that acute injections of cholinergic agonists into PRF induce PS and antagonists disrupt it, no study has charted long-term sleep-wake alterations induced by cholinergic agonists and antagonists. In this study an Alzet mini-pump is used to infuse drugs at a controlled rate (1 ul/hr or 0.5 ul/hr) over a period of 7 or 14 days.

Rats are implanted with chronic indwelling EEG and EMG electrodes and a cannula aimed at various brainstem loci. After recovery from surgery, the animals are connected to a polygraph and a 24 hr baseline record obtained. Subsequently, at 9 AM, the Alzet mini-pump is implanted and 9 consecutive (7 days drug + 2 days post-drug) 24 hr EEG recordings follow. Two weeks later a 24 hr post-experimental recording is obtained. Since this is a repeated design all comparisions are to the animal's own baseline.

Midline infusions of saline (n=4) into the PRF produce no alterations in either total sleep time (TST), slow wave sleep (SWS) or PS. Carbachol (7 days; 0.5 ug/hr) into PRF (n=5) does not alter TST, SWS or PS during day. In the night, however, carbachol significantly increases TST (+30%), SWS (+23%), and PS (+94%). The increase in PS is due to a 129% increase in PS episodes rather than change in PS duration; SWS episodes only increase 15%. Similar infusions into the bulbar region (n=4) produce no alteration during the day but induce a significant insomnia during the night cycle. 14 day carbachol (n=5) infusions into areas slightly caudal to PRF produce a selective PS augmentation for 10 days (+36%) during day, with no change in the night. Scopolamine (n=4; 7 days; 9.0 ug/hr) into the PRF selectively reduces PS only during the day (-64%). This is due to a 50% reduction in PS episodes and a 25% reduction in PS duration; no rebound is seen. In all groups the sleep-wake cycle returns to baseline levels during post-drug and post-experimental days.

These data: (1) replicate our previous findings (Neurosci.Abstr. 6: 1980), (2) extend the results in that PS can be augmented for at least 10 days, and (3) indicate that changes in PS might be due to alterations in a pontine PS trigger.

242.18 PERGOLIDE HAS BIPHASIC EFFECT ON SLEEP IN RATS. R. S. Miletich and M. Radulovacki. Univ. of Illinois College of Medicine, Chicago, IL 60612.

We have recently shown that a high dose of pergolide, a potent dopamine receptor stimulant, increased wakefulness and suppressed sleep in REM sleep deprived rats (1). These effects were abolished by pretreatment with dopamine receptor blockers indicating that sleep suppression by pergolide was done by stimulation of dopamine receptors. The present study tested the effects of pergolide on sleep in normal rats in a dose related manner.

Rats were implanted for EEG and EMG recording. Animals were divided in 4 groups and after receiving saline and pergolide (0.05, 0.2 or 0.4 mg/kg) were continuously recorded for 8 h. Their EEG records were analyzed as wakefulness, slow-wave sleep (S_1 and S_2) and REM sleep.

Time(hr)		Control	.05 mg/kg	.20 mg/kg	.40 mg/kg
0-4	W	91.67	57.33	164.50**	221.83**
	S_1	39.00	48.17	30.17	7.17**
	S_2	82.50	112.83*	42.17**	10.50**
	REMS	26.50	20.33	2.00**	0.50**
4-8	W	38.17	66.50	59.33	145.33**
	S_1	57.33	50.00	56.17	16.83**
	S_2	95.17	90.83	92.00	70.67
	REMS	49.33	32.67	32.50	7.17**

All results are means.*p<.05,**p<.01

The results show that a low dose of pergolide increased S_2 while higher doses of the drug decreased it. The effect of a low dose could be due to pergolide's action on presynaptic dopaminergic receptors with subsequent suppression of dopamine release, while the effect of high doses may result from pergolide's action on post-synaptic dopamine receptors. These findings further indicate importance of dopaminergic system in regulation of sleep-wakefulness. (Supported by ONR Contract N00014-79-0420)

(1) Miletich, R.S., et al., 22 Annual Mtg. APSS, 1982

242.19 EFFECTS OF EXTENDED SLEEP DEPRIVATION IN RATS. B. Bergmann*, A. Rechtschaffen*, J. Winter* and M. Gilliland* (SPON: D.X. Freedman). Sleep Lab., Univ. of Chicago, Chicago, IL 60637.

A new method of mechanically producing sleep loss with minimal stimulation was used to deprive 8 male Sprague-Dawley rats of sleep to the point of death. The deprived rat (Drat) and its yoked control (Crat) were housed in cages whose common floor was a horizontal disk which, when rotated, forced both rats to walk in order to avoid a 3 cm deep water bath. Both rats were electrophysiologically monitored for sleep. When a Drat showed sleep onset, the monitoring computer rotated the disk until 6 sec after the Drat awoke. Rotation averaged 23% of total time with Drats averaging 4% of baseline NREM and 3% of baseline REM sleep; Crats averaged *68% of baseline NREM and 39% of baseline REM.*

Survival--Three Drats died within 5.7 to 33.4 days; 5 were sacrificed by perfusion after 5.9 to 22.0 days when imminent death was indicated by aphagia, ataxia, EEG decline, and/or hypothermia. Crats, conversely, remained groomed and healthy-looking. Survival time of Drats correlated with their REM% (r=.938, p<.001).

Stress--Although 3 of 7 Drats had gastric ulcers and all (6 of 6) had enlarged adrenals vs. cage controls (p<.001), both Drat and Crat corticosterone levels decreased during deprivation (each p< .05, combined p<.005; assay by V. Fang, PhD, Univ. of Chicago), thus indicating a defect in glucocorticoid synthesis or an increase in its catabolism associated with even partial sleep loss. (A similar result was reported in a REM-deprivation study--Mark, J. et al., Life Sci., 8:1085, 1969.) Although immune response is usually suppressed in stress, no pneumonia was seen in lung sections (evaluation by L. Wold, MD, Mayo Clinic).

Metabolism--For both Drats and Crats, food intake (Drat-p<.001; Crat-p<.05) and temperature (Drat-p<.02; Crat-p<.10) increased over baseline, implying increased energy use, while body weight decreased (Drat-p<.0005, Crat-p<.001), implying use of energy stores. Drats lost more weight than Crats (p<.05), and also had lower liver (p<.02) and spleen (p<.005) weights.

We conclude that sleep is necessary for some vital physiological function. However, even chronic partial sleep deprivation can produce defects in corticosterone metabolism, and more generally, increase the ratio of catabolic to anabolic processes. Since corticosterone enhances many catabolic processes, one possible hypothesis is that sleep loss independently enhances catabolism, and that the low corticosterone levels result from self-regulation, i.e., high catabolism may produce non-ACTH-dependent negative feedback which lowers corticosterone levels. These low levels in turn stimulate increased ACTH release, thus producing the observed adrenal hypertrophy.

242.20 REGIONAL FUNCTIONAL ACTIVITY IN THE BRAIN OF THE RAT DURING WAKE, SWS AND REM. P. RAMM* and B.J. Frost, Dept. of Psychology, Queen's University, Kingston, Ontario, K7L 3N6.

We have compared metabolic activity (MA) in 200 brain regions with the mean MA of the brain, during each of the arousal states. In some areas, MA is elevated or inhibited relative to the brain mean. Regions exhibiting marked state-dependency may be linked to sleep mechanisms or functions.

Unrestrained rats (N=23) with electrodes and jugular cannulas were injected with 25 μCI of 2-DG via the cannula, while recordings were made of EEG and EMG. Other rats (N=10) were REM-deprived before injection and recording. 45 minutes after 2-DG injection, the brains were frozen and cut. Autoradiographic optic densities (ODs), reflecting MA in the underlying tissues, were read by computerized densitometry.

Ratios were constructed between local ODs and the mean of all sampled regions (N=200) in a given animal. These relative MA (RMA) values show areas whose MA is more markedly state-dependent than that of the brain as a whole. Product-moment correlations were obtained, between the regional RMA values and the proportion of total 2-DG converted to 2-DG-6-P during each state (similar to time spent in each state). Correlations of 0.4 or better (p <= 0.02) are deemed significant. The brain means were not correlated with any state measure (r < 0.20).

During REM, the reticular core, substantia nigra, Forel's field H1, n. ruber, pallidum and subthalamic nucleus exhibit enhanced RMA. Cerebellum (r = 0.76) and ventral thalamus (r = 0.61) show decreased RMA. These effects in motor and extrapyramidal regions reflect functional reorganization during REM atonia.

SWS is associated with decreased RMA in the n. rhomboideus (r = 0.72), an area traditionally implicated in sleep generation. Other midline thalamic nuclei exhibit much weaker metabolic state-dependency. Intrinsic and specific thalamic nuclei, including the pulvinar complex (r = 0.68) and lateral geniculate nuclei (r = 0.76), show inhibited RMA during SWS. There is inhibition of RMA in cortical layer IV (r = 0.44), specific to SWS. General decrease of RMA in sensory relay regions, and major cortical projection site, reflects functional deafferentation of the brain during SWS.

Some limbic regions exhibit enhanced RMA during SWS. This is evident in the hypothalamic core, and in the ventral blade (r = -0.47), hilus (r = -0.62), and granule cell layers (r = -0.62) of the hippocampal dentate fascia. There is also enhanced RMA in the dentate granule (r = -0.67) and ventral blade (r = -0.73) regions during REM. In the regio superior, the stratum moleculare-lacunosum shows REM-specific enhancement of RMA (r = -0.53). These data are in accordance with reports that neuronal transmission in the hippocampus is state-dependent.

242.PO PGO WAVES IN RATS: THEIR RELATIONSHIP TO STATE-DEPENDENT PROCESSES OF SENSORY RESPONSIVENESS. L.S. Kaufman and A.R. Morrison
Labs. Anat., Sch. Vet. Med., Univ. of Penna., Phila., PA 19104

Extensive investigation of PGOs recorded in the pons, lateral geniculate nucleus, and occipital cortex in cats has firmly established that this activity results from endogeneously-produced reticular activation which occurs during paradoxical sleep (PS). Studies in cats by Bowker and Morrison (Brain Res., 102:185, 1976) have redefined PGOs to include also the occurrence of activity that can be evoked by a variety of sensory stimuli from the same structures in all behavioral states. We previously reported that waves which were homologous with the pontine component of PGOs in cats could be recorded from the area of the locus coeruleus (LC) in albino rats, and that the occurrence and amplitudes of auditory evoked PGOs varied with the rats' state of sleep and waking (Sleep Res., in press). Since PCPA does not alter spontaneously-occurring PGOs in rats despite significant alterations in sleep-wake behaviors (Kaufman & Morrison, Sleep: 1982, in press), we decided to study the effects of this drug on the occurrence and amplitudes of auditory-evoked PGOs in rats.

After a minimum of 2 weeks of habituation to experimental conditions, rats were injected with 376 mg/kg PCPA methyl ester. Clicks (1000-2000/day) were presented at regular interstimulus intervals of 2.6 sec in daily experimental sessions which continued throughout the peak effect of the drug. These procedures were identical to those used in non-drugged rats. Despite significant alterations in the sleep-wake cycle, evoked PGOs displayed the same state-related pattern of occurrence and amplitudes as in non-drugged rats. Responding was lowest during PS and highest after PS. High responding was also seen following arousals from slow wave sleep. As observed in the non-drugged rats, there was no habituation of evoked PGOs within or between experimental sessions. These results support our conclusion that the amplitudes and occurrence of evoked PGOs recorded from the area of the LC are related to the rats' state of sleep and waking. It is significant that this relationship persisted after PCPA when the sleep cycle was drastically altered. Aston-Jones and Bloom recently reported a similar state-dependent relationship between auditory-evoked field potentials recorded from microelectrodes in the LC in normal rats (J. Neurosci.,8:887, 1981). Similar results were also obtained by Huttenlocher (J. Neurophysiol., 12:451, 1961; EEG clin. Neurophysiol., 12:819, 1960) in the mesencephalic reticular formation of cats.

Our results and those of Huttenlocher have led us to conclude that PGOs in rats reflect state-dependent alteration of sensory input to the LC that is modulated by the reticular formation.
(Supported by NS-13110, 1 F MH08658, and Oberlin College)

243.1 EFFECTS OF ADRENERGIC AGONISTS ON CHOROID PLEXUS ORGANIC ACID TRANSPORT. L.A. O'Tuama,* T. Bohan, C.S. Kim,*, J.D. Mann, C.R. Roe,* R. Johnson, C. Sutton*. (SPON: J.N. Hayward). Dept. of Neurology, Univ. No. Carolina Sch. of Med., Chapel Hill, NC 27514 and Dept. of Peds., Duke Univ., Durham, NC 28204.

Edvinsson et al. (Exp. Neurol., 48:241, 1975) have provided evidence for a sympathetic neural influence on CSF production. This finding suggests there may also be neurogenic effects on other functions of choroid plexus (CP) epithelium, e.g., systems for solute translocation. We have studied effects of adrenergic agonists on the CP transport system for the anionic pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) (Kim and O'Tuama, Brain Res., 224:209, 1981). Young adult rabbits were killed by exsanguination or air embolus. Lateral ventricular CP were rapidly dissected, placed on ice for 5-10 min and incubated (37^{0}C; pH=7.4) in artificial CSF containing tracer doses ^{14}C(2,4-D) ± additional solutes. 2,4-D accumulation by CP was assessed as the ratio of tissue-to-medium radioactivity (T/M) at 10 min. Control T/Ms averaged 19.06 ± 10 (S.E.) (comparable to our earlier study). Addition to the medium of L(-)-epinephrine (10^{-3} M) inhibited 2,4-D uptake by 41.7%. The inhibition of uptake was dose-dependent (23.6% of controls at 10^{-5} M). A more pronounced, dose-dependent inhibition was found with (±-)-isoproterenol (58.5%, 47.5% and 24.8% of controls at 10^{-5} M, 10^{-6} M and 10^{-7} M, respectively). Time course experiments indicated that the isoproterenol-induced change began within 1 min and was complete within 2 min after exposure of CP to the agonist. The interaction of the neurotransmitters with the CP transport system seemed markedly dependent on the time elapsing between CP removal and its exposure to radiolabel; in experiments where all CP sections were placed on ice for 2 min only before incubation, isoproterenol produced enhancement of 2,4-D accumulation (+86.6% over control values at 10^{-6} M). Experiments to investigate blockade of the agonists' effects on CP transport by propranolol will be presented. Taken in conjunction with recent in vivo studies (Lindvall et al., Brain Res., 223:160, 1981), these results suggest the possibility of selective neurogenic modulation for discrete transport pumps housed within brain barrier systems. Further study of such interactions may give new insights into pathogenesis and more selective treatment of several congenital encephalopathies.
Supported by USPHS grant NICHHD HD-03110.

243.2 BIOCHEMICAL EVIDENCE FOR CHOLINERGIC INNERVATION OF CEREBRAL CAPILLARIES. Diana N. Krause and Carmen Estrada*. Division of Neurosciences, City of Hope Research Inst., Duarte, CA. 91010

Recently we found specific muscarinic cholinergic receptors and choline acetyltransferase (ChAT) present in a heterogeneous fraction of isolated intracerebral blood vessels (JPET 221:85, 1982). We now report that these cholinergic parameters also are associated with a purified capillary fraction isolated from bovine brain. Capillaries were obtained from gently-homogenized grey matter using a sucrose gradient followed by seiving through a series of nylon meshes (149µm and 53µm) which separated the capillaries from the larger intracerebral vessels. The capillaries appeared quite pure by morphologic and marker enzyme criteria. The specific antagonist ^{3}H-quinuclidinyl benzilate (QNB), 5µM, was used to label muscarinic receptors in a crude membrane fraction of the capillaries. Significant binding was found in capillaries isolated from cerebral cortex (101 fmol/mg protein) caudate nucleus (107 fmol/mg) and cerebellum (94 fmol/mg). The lack of significant differences in binding to capillaries among the three regions is in contrast to the significant differences seen among ^{3}H-QNB binding to the grey matter of these three regions and argues against the possibility that the capillary binding represents contamination by grey matter. ChAT activity, which reflects acetylcholine synthesis and is used as a marker for cholinergic terminals, also was found associated with the capillaries from the same three brain regions. Capillary ChAT was highest in the caudate (475 pmol/mg protein/min) and lowest in the cerebellum. Cerebral capillary endothelial cells also were isolated according to Williams et al (J. Neurochem. 35:374, 1980). In this method, collagenase is used to remove the basement membrane as well as other capillary associated elements (pericytes, adhering astrocytic endfeet and possible nerve terminals) from the endothelial cells. ^{3}H-QNB binding to crude membranes prepared from the isolated endothelial cells was not significantly different from that found with the more intact capillary preparation. However, ChAT activity was significantly reduced in the collagenase-treated preparation. These data suggest that specific muscarinic receptors are present in cerebral capillary endothelial cells and may be innervated by cholinergic nerve terminals. A cholinergic system at the capillary level could be important in regulating blood-brain barrier function. (Supported in part by USPHS grant NS 12116).

243.3 HYDRAULIC CONDUCTIVITY OF BLOOD-NERVE BARRIER IN FROG SCIATIC NERVE. S. Odman,* M.E. Michel,* P.J. Robinson,* P. Ask,* H. Levitan, S.I. Rapoport (SPON: R.M. Steinman). Laboratory of Neurosciences, National Institute on Aging, Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224.

Edema and dehydration of the peripheral nervous system is a function of fluid flow through the tissues which bound it. The endoneurial space of vertebrate peripheral nerves is bounded by a nerve sheath or perineurium and the blood-nerve barrier (BNB) consisting of endothelial cells surrounding capillaries. The rate of fluid flow into and out of this space is a function of the hydraulic conductivity (L_p) of the bounding tissues. Although L_p and the elastic properties of the perineurium have recently been measured (Ask et al., Am. J. Physiol., in press), there is no information on the L_p of the BNB. We have measured capillary surface area, changes in nerve diameter and endoneurial pressure as a function of the osmotic pressure in the capillaries, and developed a mathematical model relating these parameters to calculate hydraulic conductivity of the BNB. The number and dimensions of the capillaries in a nerve segment were determined from light micrographs. The endoneurial pressure was measured with a micropipette inserted through the perineurium and connected to a null-balance micropressure instrument. A polyethelene tube was inserted into an iliac artery through the aorta, and capillary osmotic pressure was changed by adding albumin, sucrose, or NaCl to normal Ringers, and perfusing at rates from 0.014-0.82 ml/min with a perfusion pump connected to the catheter. The 13 ± 1 (mean ± S.E., n = 10) capillaries per nerve occupied 3.0% ± 0.3% of the volume of the nerve segment and had a surface area/unit length which was 56% ± 2% that of the perineurium. The capillary surface area was not a function of perfusion rate. When albumin was used as the perfusate a hydraulic conductivity of (9.0 ± 4) x 10^{-11} cm^{3}s^{-1} dynes^{-1}(n = 14) was calculated. From this L_p, and assuming a reflection coefficient of 1 for albumin, we calculated BNB reflection coefficients for sucrose and NaCl of (8 ± 5) x 10^{-2} and (2 ± 1)x 10^{-2} (n = 14), respectively. Such a relatively "leaky" epithelium must represent the primary site for transfer of both solvent and solute into and out of the endoneurial space in the frog.

243.4 ULTRACYTOCHEMICAL STUDIES OF THE VESICULAR AND CANALICULAR TRANSPORT SYSTEMS IN THE ALTERED MAMMALIAN BLOOD-BRAIN BARRIER A.S. Lossinsky*, A.W. Vorbrodt* and H.M. Wisniewski*
NYS Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y., U.S.A.

Following blood-brain barrier damage, increased permeability to horseradish peroxidase (HRP) manifests in the formation of pinocytic vesicles and tubulo-canalicular structures (TCS). Both types of structures have been considered to be engaged in the increased transport of macromolecules across the endothelial cell (EC). We wanted to determine the origin of the two types of EC structures following injury to the BBB. Mice and cats were subjected to either traumatic or cold lesion brain injuries respectively. HRP (Sigma II) was intravenously injected following brain lesions (0.15 mg/mouse; 600 mg/cat) which circulated for 1-2 hours prior to perfusion with aldehydes. Alkaline phosphatase (AP) was employed as an enzymatic marker of EC plasmalemma. Sections of coronal brain slices were incubated for cytochemical detection of HRP and AP and processed for electron microscopy. Fine structural analysis of leaking micro-blood vessels demonstrated pinocytic vesicles and deep invaginations of the luminal plasmalemma which seemingly transformed into tubular profiles. Because the limiting membranes of all transport structures showed a positive reaction for AP, we conclude that vesicles and tubules originate in ECs from luminal plasmalemma constituting the exoplasmic membranous system of 100 Å thickness.
Financial support NINCDS Grant NS 18079.

243.5 ENTRY OF EXOGENOUS ASPARTATE INTO CIRCUMVENTRICULAR ORGANS BUT NOT OTHER REGIONS OF BRAIN. M.T. Price, M.E. Pusateri*, O.H. Lowry*, and J.W. Olney. Depts. of Psychiatry and Pharmacology, Washington University Sch. Med., St. Louis, MO 63110.

We previously reported that 2 g/kg Asp injected subcutaneously (sc) into 4 d old mice resulted in 1) a 200-fold elevation of serum Asp, 2) a selective uptake of Asp by neurons in circumventricular organs (CVO), brain regions which are thought to lack blood brain barriers, and 3) extensive neuronal damage in CVO. We have now administered Asp to adult mice (which are less prone than neonates to Asp-induced CVO damage) in 2 sc injections 30 min apart, each injection containing 1/2 of the total dose. The total doses ranged from 0.12-1.0g/kg which is 6-50% of a minimum toxic dose. Within 30 min of the second injection of 0.25-0.5g/kg Asp, serum levels increased 50 x. Asp concentrations rose to 2.5 x normal in the subfornical organ (SFO) and to 2.5-3 x in the arcuate-median eminence of hypothalamus (AH-ME). When only 0.06-0.13g/kg doses of Asp were given, serum levels rose 4 x, SFO was normal while AH-ME Asp rose 1.5 x. In all of these animals, Asp in the striatum and in the ventromedial nucleus (VM), just adjacent to AH-ME, were unaffected. In most animals, sampling in each nucleus was random but in 2 animals from the 0.25-0.5g/kg dose group, and in 2 saline-treated animals, the AH-ME-VM region from a 20 μm thick section was divided into subregional samples (50 μm x 50 μm) and an "Asp map" constructed. In Asp dosed animals ME was 2.5 x control levels, ventral AH 1.7 x, dorsal AH 1.5 x, transitional zones (T) between AH & VM 1.1 x, and VM 0.9 x. Thus the increases in Asp concentration diminished in a gradient from ME to VM. Since ME contains fenestrated capillaries, but AH, T and VM do not, we propose that sc Asp gained egress through ME capillaries and diffused into adjacent AH. Asp levels fell abruptly at T, a neuron-free glia enriched zone which comprises an outer border separating damaged from undamaged tissue zones in animals subjected to toxic doses of Asp. Previously we reported that subtoxic sc doses of N-methylaspartate (NMA), an Asp analog, results in release of luteinizing hormone (LH). This effect is thought to stem from NMA-induced activation of AH neurons since it does not occur in animals that are deficient in AH neurons (due to glutamate treatment in infancy) and NMA does not stimulate LH release from the in vitro pituitary. Our findings support the hypothesis that fenestrated capillaries in CVO brain regions permit entry of acidic amino acids such as glutamate, Asp and NMA into CVO brain regions thus exposing CVO neurons to the excitatory and toxic actions of these agents. Supported by grants from Amer. Cancer Soc. BS 4x; NIH, NS08862 (OHL); NS 09156; RSA MH 38894 (JWO).

243.6 REGIONAL BLOOD VOLUME OF RAT BRAIN AS DETERMINED BY DIFFERENT SIZED MOLECULES. Y.Z. Ziylan,* Q.R. Smith and S.I. Rapoport. (SPON: H. Levitan) Laboratory of Neurosciences, Gerontology Research Center, National Inst. on Aging, Baltimore, MD 21224.

Cerebrovascular permeability in conscious rats can be determined from the initial uptake of a radiotracer by brain, following the i.v. injection of tracer (Ohno et al., Amer. J. Physiol. 235: H299, 1978). To calculate the concentration in the brain parenchyma, total brain radioactivity is corrected for intravascular activity, which is obtained from the product of brain vascular volume and blood concentration. An accurate measurement of residual brain blood volume is essential for sensitive determination of cerebrovascular permeability, as the vascular contribution often is a significant fraction of the total brain radioactivity. The purpose of this study was to determine which radiotracers provide an accurate measurement of regional brain blood volume.

The regional vascular volume of Osborne-Mendel rats was determined from the brain distribution, as a function of time, of radiotracers of different sizes and molecular weight. Radiotracers were chosen which are known or expected to enter the brain very slowly. They were ^{14}C-Sucrose (mol. wt. 340), ^{3}H-inulin (mol. wt. 5500), ^{3}H-dextran (mol. wt. 7000-12000), and ^{51}Cr-transferrin (mol. wt. 80,000). At 1 and 5, 30, or 60 min. after a tracer was injected i.v., the animals were decapitated and samples of 14 brain regions, of arterial blood, and of plasma were analyzed for radioactivity. Results were expressed as a tissue space (%) = 100 x (dpm/g brain)(dpm/ml blood).

Taking the frontal cerebral cortex (FCC) as a representative region, the 1 min space was comparable for sucrose (1.5%), inulin (1.4%), and dextran (1.3%). Five min after injection, the sucrose and inulin spaces of the FCC increased to 2.4% and 1.9%, respectively, whereas the dextran space remained constant (1.2%). Between 5 and 30 min after injection the inulin space (2.0%) and dextran space (1.2%) did not change significantly, while the sucrose space increased to 3.4%. The constant space between 5 and 30 min for inulin and dextran is consistent with a cerebrovascular PA $< 10^{-7}$ sec^{-1}; the increase in the sucrose space is consistent with a significantly greater PA of the blood-brain barrier to sucrose. The ^{51}Cr-transferrin space (1.0%) was comparable through slightly less than that of dextran and did not change signficantly between 5 and 60 min. A 2-fold regional variation was noted in blood space among different regions, the largest space was found in the olfactory bulb and the smallest space in the caudate nucleus. In conclusion, nonelectrolytes with a mol. wt. < 6000 (sucrose, inulin) appear to have a larger blood volume in the rat brain than do dextran or transferrin. This discrepancy may arise from the exlusion of macromolecules from the capillary extracellular matrix.

243.7 THE EFFECT OF OCTANOIC ACID ON THE UPTAKE OF 5-HYDROXYINDOLEACETIC ACID BY RABBIT CHOROID PLEXUS. C.S. Kim*, L.A. O'Tuama*, C.R. Roe* and J.D. Mann (SPON: J.M. Lauder). Dept. of Neurology, Biol. Sci. Res. Ctr. and Pediatrics, University of North Carolina Sch. of Med., Chapel Hill, NC 27514 and Duke Univ. Med. Ctr., Durham, NC 27706.

The relationship between the encephalopathy of Reye's syndrome and the elevation in serum octanoic acid found in those patients is not understood. Accumulation of endogenously produced organic acids in cerebrospinal fluid (CSF) and brain, secondary to reduced clearance by choroid plexus (CP), could be a contributing factor in the development of encephalopathy. Active transport of 5-hydroxyindoleacetic acid (5-HIAA), the organic acid metabolite of serotonin, has been described in the choroid plexus, but not in cerebral cortex (Forn, Biochem. Pharmacol. 21:619-624, 1972). The present in vitro study was undertaken to determine whether octanoic acid interferes with CP uptake of 5-HIAA.

Young albino rabbits of either sex were sacrificed by decapitation. CP was collected from the lateral and fourth ventricles and incubated for either 10 minutes or 60 minutes in artificial CSF containing ^{14}C-5-HIAA. When the incubation medium contained either 0.1 mM or 1.0 mM octanoic acid, choroid plexus uptake of 5-HIAA at 10 minutes was reduced by 44% and 54% respectively in fourth ventricle CP ($p < .05$). With the same levels of exposure to octanoic acid, lateral ventricle choroid plexus uptake of 5-HIAA was reduced by 40% and 63% ($p < .01$). Carrier mediated, concentrative transport of 5-HIAA by CP continues during more prolonged incubation. Tissue to medium ratios of 4.3 $\pm$ 0.7 (S.E.) and 6.3 $\pm$ 1.2 were achieved in fourth and lateral ventricle CP respectively when incubations were continued for 60 minutes in control media. The ratios fell to 2.3 $\pm$ 0.3 and 2.8 $\pm$ 0.4 when incubations were carried out in media containing 1.0 mM octanoic acid ($p < .05$, $p < .01$).

In an earlier study, we demonstrated that octanoic acid strongly inhibits the transport of the organic acid herbicide 2, 4-D via the organic acid transport system in CP (Kim et al., Ped. Res. 16:336, 1982). The present study demonstrates that octanoic acid also inhibits CP uptake of the endogenous acid metabolite 5-HIAA. Failure to clear this compound from CSF by choroid plexus could contribute to the development of encephalopathy in association with elevated levels of octanoic acid systemically.

243.8 CEREBROSPINAL FLUID DYNAMICS IN THE RAT: ANALYSIS OF STEADY-STATE RESPONSES TO SINUSOIDAL INPUT. J.D. Charlton*, N.E. Pederson*, R.N. Johnson and J.D. Mann. Dept. of Neurology and Biomedical Engineering, Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514.

A majority of previous studies of cerebrospinal fluid (CSF) dynamics have employed analyses of responses to bolus, constant rate or constant pressure inputs into the CSF compartment. In the present study, we analyzed CSF pressure responses to sinusoidal variation in the infusion rate.

Adult albino rats were anesthetized with halothane (1% in 0_2), paralyzed with pancuronium bromide and artificially ventilated. Body temperature was maintained at 37°C and arterial blood pressure was monitored via a femoral artery catheter. A spinal needle was stereotaxically placed in the cisterna magna for infusion of artificial CSF and simultaneous intracranial pressure monitoring. Infusion of artificial CSF through the spinal needle was controlled by a Sage infusion pump modified for external voltage control. The pump was driven by either an offset sinusoidal signal, or a constant voltage signal, adjusted so that the infusion rate was in the range of zero to 40.0 ul/min..

The sinusoidal variation in intracranial pressure was recorded on a strip chart recorder simultaneously with the infusion rate signal. The two signals were analyzed for peak-to-peak variation, mean value, and phase shift for input frequencies in the range of .003 to .01 Hz. The system was modeled as a parallel resistance and compliance at each mean infusion rate. The resistance to CSF absorption as a function of pressure was determined as the change in pressure divided by the change in infusion rate. The compliance was then obtained at each mean pressure from the frequency dependent phase shift between input and output using a first order linear model. Reistance values were lower for higher average infusion rates, with compliance decreasing at higher mean intracranial pressures.

These findings are consistent with our previous work (Mann et al., Ann. Neurol. 3:156-165, 1978; Johnson et al., T.I.T.J. Life Sci. 8:79-82, 1979), showing a non-linear decrease in both resistance to CSF absorption and system compliance with increasing pressure. The technique described here offers a viable alternative to constant rate and constant pressure infusion techniques for CSF system analysis and modeling. It allows more rapid assessment of intracranial compliance and resistance and thus may prove to be a useful investigative technique.
Work supported by NIH Grant NICHD-1443.

243.9 CHANGES IN BLOOD-BRAIN BARRIER FUNCTION ASSOCIATED WITH CONDITIONED FEAR IN RATS. C. M. Pechura*, L. R. Watkins, J. T. Povlishock, D. P. Becker and R. L. Hayes. Depts. of Physiology, Anatomy and Neurosurgery, Med. Col. of Virginia, Richmond, VA. 23298.

It has been widely reported that the blood-brain barrier (BBB) can be disrupted by the induction of pathophysiologic events such as hypertension and intravenous infusion of hyperosmotic agents. Although recent studies have shown the barrier to be affected by the antidepressant, amitriptyline (Preskorn, S. H., et al., Science, 213:469, 1981), and under the modulation of circadian rhythms (Johansson B., and Martisson L., Acta Neurol. Scand., 62:96, 1980) in both studies barrier function was assessed following physiological challenges such as hypercapnea and hypertension. In contrast, the present study sought to assess the response of the BBB to the environmentally relevant state of classically conditioned fear.

For each of 3 days prior to testing, 20 male, Sprague-Dawley rats (350-450g) were placed in a standard plexiglas operant chamber with a grid floor through which 0.5 mA of scrambled shock was delivered for 10 sec. On the 4th day, experimental animals (N=12) were placed in the chamber in the presence of all other stimulus cues but were not shocked. 1 day prior to testing, cannulae were placed in the external jugular vein and externalized on the animals' back under methoxyflurane anesthesia. On the 4th day, experimental animals were injected intravenously with 75-100 mg/kg horseradish peroxidase, HRP, (Sigma VI) 5 min prior to placement in the operant chamber. Control animals (N=8) were injected with HRP in the animal colony room. At 40 min post HRP injection animals were deeply anesthetized with sodium pentobarbital (i.v.) and perfused with aldehydes. The brains were sectioned at 50 μ and processed for visualization of HRP.

Extravasation of peroxidase was more prominent in brain sections from experimental animals in contrast to control sections. This extravasation was most often seen in cortical regions, hippocampus and dentate gyrus, as well as ventral regions of the medulla. HRP reaction product was less frequently visualized in the inferior colliculi and areas dorso-lateral of the hypothalamus. These data suggest that the physiological components of an emotional state such as fear may include altered function of the blood-brain barrier. Supported by NIH Grant #NS-12587.

243.10 A VASCULAR-SPECIFIC MONOCLONAL ANTIBODY. G.R. Dutton, A.L. Gard, and F.P. White. Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, IA 52242.

We have used a continuous cell line (Z-cytes) derived from cerebral microvessels as an immunogen to produce monoclonal antibodies which specifically bind to vascular surfaces. Spleen cells from mice immunized against Z-cytes were used to prepare lymphocyte hybridomas secreting monoclonal antibodies. A washed, crude membrane fraction prepared from the Z-cytes was used as a target to screen the hybridomas using an ELISA technique. The cell types recognized by the various antibodies were localized by indirect immunofluorescence.

One of the hybridomas (3G7) produced an antibody which bound specifically to microvessels. In primary cultures of rat cerebellum a sub-population of flat cells (< 10%) showed surface staining. This sub-population consisted of neither neurons nor astrocytes, as determined by double labelling with antibodies to GFAP and tetanus toxin. The antigen recognized by 3G7 was found in high concentration on the surface of Z-cytes. Also, 3G7 labelled kidney glomeruli, and the microvasculature in heart, liver, and kidney of rats of various ages. However, the brain microvasculature was not labelled in adult rats. Labelling by 3G7 was found only in the brains of animals younger than three weeks of age.

These results suggest that an antigen localized to either the endothelial cell or its basement membrane is postnatally regulated in the brain, but not in the other organs tested.

This work was supported by USPHS grant NS 17666 (G.R.D.), training grant GM 07069, and by MRC Canada grant MT 5405 (F.P.W.).

243.11 A SAMPLE-PLUG TECHNIQUE FOR MEASURING LUMINAL ENDOTHELIAL CELL MEMBRANE FLUIDITY IN RAT CORTICAL CAPILLARIES. D.G. Lange, C.S. Lai* and C.W. Christensen. National ESR Center, and Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

Using a newly developed sample-plug technique (SPT), it is now possible to measure the in vivo membrane fluidity of the luminal surface of rat cortical capillaries. Following bilateral carotid artery perfusion with the spin-labeled fatty acid 5-doxyl stearic acid (5-DS), 1 mM at 1.49 ml/min for 5 min, the rat cortex is dissected free, and a 200 mg sample-core is taken with a Pasteur pipette. The pipette is then placed into the cavity of an ESR spectrometer and a spectra reflecting the lipid environment of the luminal surface of the capillary endothelial cell is obtained. Both order parameter (S) values and spin-label reduction rates (SLRR) can be calculated. The S value for SPT (0.682 ± 0.004 N=4) is comparable to that obtained from capillaries isolated from grouped cerebral cortices (6-8 animals), which had been previously perfused with 5-DS (S value = 0.680 ± 0.005). Similarly, comparable values for SLRR are determined for both SPT and isolated capillaries (t 1/2 approximately 12 min). The major advantage of this new technique is its specificity and increased sensitivity (approximately 50x greater than with capillary isolation). In addition, the rapidity of the SPT procedure makes it a highly desirable improvement over the previously employed capillary isolation technique. One significant disadvantage of the SPT procedure is its inability to measure extra-luminal lipid characteristics, which can be accomplished by an in vitro application of 5-DS, to previously isolated capillaries. Thus, it is now possible to measure with SPT rapid and transitory changes in luminal lipid membrane characteristics of rat cortical capillaries in discrete regions of brain parenchyma. (Supported by PHS Grants ES 02006 and RR 01008).

243.12 BLOOD-BRAIN BARRIER PERMEABILITY TO WATER MEASURED BY A DUAL-ISOTOPE, SINGLE-TRANSIT TECHNIQUE: EFFECT OF ALTERED PLASMA OSMOLALITY, G. H. Irwin, D. L. Stewart*, M. Reimold*, J. Crumpacker*, M. Kruse*, and S. H. Preskorn, Dept. of Biol., Washburn Univ., Topeka, KS 66621.

We have previously reported a technique for determination of the effective permeability of the blood-brain barrier to water (PwS), in the rat (J. CBF and Metabol. 1:S391, 1981). Single-transit uptake of 3H-labeled water (Ew) is measured using 14C-labeled butanol. Butanol serves simultaneously for measurement of cerebral blood flow (CBF) by indicator fractionation. PwS is calculated as PwS = -CBF * ln(1-Ew) after corrections for incomplete retention of the butanol tracer have been applied (Irwin and Preskorn, Brain Res., In Press). PwS measured in this way is increased by central adrenergic agonists (Sci. 213(4506):469). We now report the effect of plasma osmolality (pOs) on PwS.

Rats were curarized and passively ventilated with N2O/O2. Hypo- and hyper-osmotic conditions were achieved by I.P. infusion of either distilled water or 0.6M mannitol (200 ml/Kg body weight) 10 minutes prior to Ew and CBF measurement. Final pOs was: 304+6 mOsM/L (control); 280+7 mOsM/L (water); and 321+8 mOsM/L (mannitol). In a parallel study, rats pre-treated with the alpha-adrenergic blocking agent, phenoxybenzamine (PBZ, 25mg/Kg, I.P., 18 hours in advance) were given no osmotic stress or were made hyper-osmotic with mannitol. Treatment classes contained 18 to 22 animals.

The hypo-osmotic condition had no effect on PwS or CBF response to CO2. Hyper-osmolality inhibited CBF autoregulation and produced a marked increase in PwS. Adrenergic blockade with PBZ had no effect on CBF or PwS, nor did PBZ pre-treatment antagonize the hyper-osmotic suppression of autoregulation. However, PBZ did reverse hyper-osmotic increase in PwS.

Our finding of increased PwS and decreased CBF correlates with the effects of centrallly-administered vasopressin (Raichle and Grubb, Brain Res., 143:191, 1978) but not with the increase in CBF which accompanies classical "osmotic barrier opening" (Raichle, et al., Fed. Proc., 36:470, 1977). Inhibition of the PwS effect by alpha-adrenergic blockade may correspond to known adrenergic innervation of vasopressinergic portions of the hypothalamus.

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243.13 ACIDOSIS AND PENETRATION OF FLUORESCEIN AND OTHER TRACERS THROUGH THE RAT RETINAL PIGMENT EPITHELIUM. N.L. Shinowara, P.A. Grimes,* S.I. Rapoport and A.M. Laties.* Lab. of Neurosciences, GRC, Natl. Inst. on Aging, NIH, Baltimore MD 21224 and Dept of Ophthalmology Univ of Pennsylvania School of Medicine, Phila PA 19104

Since fluorescein permeability was reported to increase across retinal pigment epithelia (RPE) of hypercapnic rats (Rapoport, Fredericks, Laties; Exp. Eye Res. 30: 129-141, 1980), horseradish peroxidase (HRP), fluorescein, carboxyfluorescein and Evans blue have been used to examine RPE permeability during hypercapnia and normocapnia. Partially restrained, awake Osborne-Mendel adult rats breathed either air (control) or 25% (v/v) CO_2 in air for 1 hr, after which the animals were anesthetized and enucleated. Blood pressure was monitored. An arterial blood sample was obtained 5 min before the end of the experiment, for measurement of pH, $PaCO_2$ and PaO_2. Mean values (mm/Hg + S.E.M.) for arterial blood pH, $PaCO_2$ and PaO_2 were 7.41 + 0.03, 38.5 + 1.2 and 85.5 + 4.0 for controls and 6.74 + 0.02, 222.5 + 15.0 and 116.9 + 7.8 for hypercapnic rats. Tracers were injected i.v. and allowed to circulate for 1 to 20 min before enucleation. Tissue with HRP was fixed, reacted with diaminobenzidine and prepared for light and electron microscopy. Eyes with fluorescent tracers were rapidly frozen, freeze dried and prepared for fluorescence microscopy. After 1 to 20 min HPR did not cross the RPE during hypercapnia or normocapnia. RPE tight junctions remained functionally intact to the HRP. Similarly, 15-19 min of Evans blue or 1-20 min of carboxyfluorescein circulation revealed no tracer penetration through the RPE. However, after 1 min of circulation, fluorescein penetrated the RPE and entered the neural retina in all hypercapnic rats, but not in controls. To examine the effect of metabolic acidosis on fluorescein permeability, NH_4Cl was infused i.v. to lower blood pH to 7.18 + 0.02 from 7.37 + 0.01. $PaCO_2$ was not elevated. After 1 min of circulation, fluorescein penetrated the RPE and neural retina of all acidotic animals. The staining of the RPE increased as the blood pH declined. These results show that lowering blood pH by either CO_2 or NH_4Cl enhances the passage through the RPE of intravascular fluorescein, but not of the other tracers, including carboxyfluorescein which is comparable in molecular weight and fluorescence intensity to fluorescein. At acid pH fluorescein has an increased proportion of monoanion, is more lipid soluble than carboxyfluorescein (Grimes, Stone, Laties, Li; Arch. Ophthalmol. 100; 635-639, 1982) and is more permeable through the RPE. In conclusion, barrier function of the RPE tight junctions remains intact to HRP, Evans blue and carboxyfluorescein during hypercapnia. During acidosis the increased transepithelial penetration of fluorescein is due, in part, to pH dependent changes in fluorescein lipid solubility.

243.14 A NEW METHOD TO DETERMINE CEREBROVASCULAR PERMEABILITY IN THE ANESTHETIZED RAT. Y. Takasato*, Q.R. Smith and S.I. Rapoport. Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, MD 21224.

We have developed an in vivo rat brain perfusion technique to study the transport of solutes across the blood-brain barrier (BBB). This method allows the quantitative measurement of brain uptake for solutes with a BBB permeability greater than or equal to that of sucrose. Several kinds of perfusate solutions may be used (modified bicarbonate-Ringer, artificial plasma, artificial blood, or whole blood), in which specific solute concentrations can be controlled. Furthermore, this method is not subject to errors in the measurement of solute uptake into the brain due to radiotracer biotransformation by tissues other than the brain (e.g. liver).

A cerebral hemisphere was perfused by retrograde infusion of a controlled solution into the external carotid artery. Briefly, Osborne-Mendel rats were anesthetized with Na pentobarbital. The right pterygopalatine artery was ligated and the external carotid artery was catheterized. Several types of perfusate were examined. Each contained one of 4 [14-C]-radiotracers (urea, glycerol, trimethylene glycol, or cycloleucine) and [3-H]-dextran. Solutions were oxygenated with 95% air/5% CO_2 and equilibrated at 37°C. One sec before the initiation of perfusion, the right common carotid artery was ligated. After 1 min of perfusion at a rate of 1.6 ml/min, the rat was decapitated and samples from 12 brain regions and infusion fluid were analyzed for radiotracer content. The cerebrovascular permeability-area product was calculated from the equation: $PA = C_{brain}/C_{plasma} \times t$.

Following 1 min of perfusion, the integrity of the BBB was checked by the brain uptake after i.v. injection of [3-H]-sucrose; no significant difference was found between perfused and non-perfused animals. Using the perfusion technique, the cerebrovascular PA's of two non-metabolized solutes, urea and cycloleucine, equaled the respective PA's obtained with the i.v. injection technique (Ohno et al., Amer. J. Physiol. 235: H299-H307, 1978). The PA for urea, which is transported passively, and for cycloleucine, which is transported by carrier-mediated diffusion at the BBB, were found to be 0.5-0.8 x 10^{-4} and 2-3 x 10^{-4} sec^{-1}, respectively. The PA for glycerol, 0.4-0.8 x 10^{-4} sec^{-1} and the PA for trimethylene glycol, 6-8 x 10^{-4} sec^{-1}, were about one-tenth their respective values as determined with the i.v. injection method. Because glycerol and trimethylene glycol can be metabolized rapidly after i.v. injection, we suggest that the difference in PA for these compounds using the perfusion and i.v. methods is due to metabolism to more-permeable solutes such as glucose.

243.15 BIOGENIC AMINE DEPLETION ALTERS CEREBROMICROCIRCULATION. J. Solnick*, T. Kent*, S. Preskorn, G. Irwin, Depts. of Psychiatry & Pharmacology, Univ. Kansas Med. Ctr., Kansas City, KS 66103.

Central adrenergic neurons have been implicated in the regulation of: 1) cerebral capillary permeability to diffusion-limited substances (P), 2) the surface area of the capillary bed (S), and 3) cerebral blood flow (CBF). Direct stimulation of this system increases the PS product and has variable effects on CBF. Administration of CO_2 stimulates the firing rate of these neurons and produces increases in both PS and CBF. We tested the possibility that the central adrenergic neurons are involved in mediating in part the CO_2-induced changes in PS and CBF by pretreating animals with either tetrabenazine (TBZ)--which inhibits vesicular storage of biogenic amines--or 6-hydroxydopamine (6-OHDA). A dual label isotope procedure (Irwin and Preskorn, Brain Res., 1982) was used to simultaneously measure CBF and PS to water. Rats were passively ventilated with O_2, NO_2 and varying amounts of CO_2. Forty-five minutes after 20 mg/kg of TBZ i.p., CBF and PS were measured in the left forebrain. TBZ treated animals showed increased responsiveness of CBF to CO_2, with no change in the responsiveness of PS to CO_2. Total levels of norepinephrine (NE) and dopamine (DA) were found to be 90% depleted in the right forebrain using high performance liquid chromatography with electrochemical detection. NE alone was depleted (70%) by intracerebroventricular injection of 125 ug 6-OHDA preceded by i.p. injection of 50 mg/kg buproprion, a dopamine reuptake blocker. After two weeks, CBF and PS were measured: CBF was decreased and PS was increased as a function of CO_2. Neither TBZ nor 6-OHDA affected pH, arterial O_2 or blood pressure. Our results support the role of central NE in the regulation of CBF and PS. Moreover, an effect on P itself is suggested since PS did not change in the same direction as CBF as would be expected if P remained constant and S paralleled CBF due to recruitment or vasodilitation. Both TBZ and 6-OHDA depleted total brain stores of NE but had different effects on PS and CBF. This apparent paradox may be due to TBZ-induced acute release of stored NE as suggested by in vivo electrochemistry. Other possibilities are: 1) TBZ affects all biogenic amines and 2) compensatory changes may have occured in the 6-OHDA treated animals due to the two week recovery period. These possibilities are being examined by pharmacological and lesioning experiments. (Supported by MH-00272 and NS-17252).

	CBF vs $PaCO_2$	PS vs $PaCO_2$	N
Control	y= .75+.035X r=.83	y=1.94+.019X r=.65	49
TBZ	y=-.89+.073X** r=.86	y=1.22+.028X r=.79	17
6-OHDA	y=.068+.034X** r=.78	y=1.62+.033X* r=.73	16
ANOVA; ** p<.01 vs Control; *p<.05 vs Control			

244.1 TACTILE DISCRIMINATION AND SOMATOSENSORY EVOKED RESPONSES DURING ELECTRICAL STIMULATION IN MEDIAL LEMNISCUS OF RAT. M. P. Campos-Domingo* and G. P. Frommer. Dept. Psychology, Indiana Univ., Bloomington, IN 47405.

Monkeys, cats, and rats fail to show deficits in tactile discrimination following subtotal lesions in the midbrain medial lemniscus (ML) (Schwartz et al., Exp. Neurol., 1972, 37, 582-596; Frommer, Exp. Neurol., 1981, 73, 775-800). We here show that tactile discrimination is disrupted in rats by electrical stimulation at this site.

Nine female albino rats were anesthetized, blinded, and implanted bilaterally with electrodes aimed at midbrain ML and in the forepaw area of somatic cortex I. ML stimulation parameters were then established for subsequent testing using 1) effects of conditioning ML shocks (.05 msec, up to 32 V) on cortical responses to forepaw shocks (.2 msec, 20 V, X4--X8 threshold), and 2) behavioral reactions to trains of ML shocks (50/sec). The rats were then trained 5 trials/day on a series of roughness discriminations (Frommer, 1981) until the finest had been mastered ($\geq$ 14/15 correct over 3 days). Effects of electrical stimulation to ML were tested on this discrimination for 29-32 days. Each rat was stimulated (50/sec) once per day for the duration of trial 2, 3, or 4. Finally the electrophysiological effects of ML shocks were retested, and electrode tips were identified in Nissl stained sections.

Four rats showed deficits on stimulation trials (16/32 to 23/32 correct) but not on preceding or following trials (minimum of 29/32 correct)(Cochran Q, $p < .01$). These rats had electrode tips in ML, and their cortical evoked responses to forepaw shocks were attenuated bilaterally > 80% by conditioning ML shocks. Four other rats showed no depression on stimulation trials or generalized depression on preceding and following as well as stimulation trials. These rats had electrodes outside ML, and their cortical evoked responses to forepaw shocks were attenuated bilaterally < 80% by conditioning midbrain shocks. The ninth rat was intermediate between these two groups on all three measures. The % attenuation of cortical forepaw evoked responses by conditioning ML shocks correlated highly with % deficit in discrimination on stimulation trials ($r = .90$, $p < .01$).

This disruption of tactile discrimination by ML stimulation is interpreted in terms of interference with sensory information in the somatosensory system. Alternative explanations in terms of aversive properties, arousal effects, motor effects, or state dependent effects of brain stimulation cannot be supported by the data. (Supported by NIH Grant GM 29254.)

244.2 SPATIAL RELATIONS OF INDIVIDUAL MEDIAL LEMNISCAL AXONS IN THALAMIC VENTROBASAL COMPLEX OF MONKEYS. E.G. Jones, James L. O'Leary Division of Experimental Neurology and Neurological Surgery and McDonnell Center for the Study of Higher Brain Function, Washington University School of Medicine, St. Louis, MO. 63110

A recent study demonstrated three features of somatic sensory thalamocortical organization in monkeys (Jones et al., J. Neurophysiol., 46, 1982): (1) Place and modality - specific columns in the monkey first somatic sensory area (SI) of the cerebral cortex receive inputs from bundles of thalamocortical axons arising from narrow, elongated aggregations ("rods") of neurons that extend through much of the anteroposterior dimension of the thalamic ventrobasal complex. (2) Receptive field mapping in the ventrobasal complex (VB) reveals similar narrow but anteroposteriorly elongated sequences of neurons all with virtually the same receptive field and modality properties. (3) Injections of tracers in the dorsal column nuclei demonstrate elongated rodlike terminations of lemniscal axon bundles in VB.

In order to determine whether single lemniscal axons contribute terminations to all cells of a thalamic rod or only at selected levels along a rod, individual axons were studied after anterograde filling with horseradish peroxidase. The medial lemniscus was identified electrophysiologically at a level just below and behind the thalamus and the enzyme injected. The axons were demonstrated by cobalt-enhanced diaminobenzidine after a 12-24 hour survival.

Each axon enters VB vertically but commonly bends horizontally forwards before branching and ending in one or more, sagittally-compressed terminal ramifications 100-200 microns wide. Axons ending in the central core of VB devoted to cutaneous representation have terminations that are more restricted antero-posteriorly than the thalamocortical rods. These results indicate that only some cells of a cutaneous rod receive inputs from each member of the bundle of lemniscal fibers ending along it and, potentially, that the bundle of thalamocortical axons arising from a rod and ending in a cortical column could contain axons with different functional properties. Most lemniscal axons end in the cutaneous core at one dorsoventral level but a few have a further terminal formation at a second level, suggesting that a single lemniscal axon could influence more than one cortical column by ending in more than one rod. Axons ending in the anterodorsal shell of VB devoted to deep representation tend to have several terminal formations in sequence over an extended anteroposterior distance, implying that in this part of VB most cells of a rod and its cortical column receive input from all the lemniscal axons contributing to it.

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244.3 SOMATOSENSORY THALAMOCORTICAL CONNECTIONS IN THE RACCOON: AN HRP STUDY. S. Warren*, B. J. Pettit*, and B. H. Pubols Jr. (SPON: C. I. Thompson). Department of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA 17033

Representation of the glabrous surfaces of the raccoon's hand is precisely somatotopically organized in both the thalamic ventrobasal complex (VB), and primary somatosensory cortex (SmI). In VB, digital representations are found within separate subnuclei, demarcated by myelinated fiber laminae (Welker & Johnson, 1965). In SmI, each digit is represented on a separate subgyrus, glabrous surfaces on gyral crowns, hairy surfaces within demarcating sulci (Welker & Seidenstein, 1959). Additionally, it has been reported that thalamocortical afferents terminate preferentially within gyral crowns. In the present study, the retrograde transport of horseradish peroxidase (HRP) from SmI to VB was utilized to make three types of comparison: (1) injections of an entire gyral crown versus more discrete injections; (2) injections of gyral crowns versus sulcal fundi; (3) injections into SmI regions versus the central sulcus homologue.

HRP (Sigma VI, 30-40% solution) was injected (0.1-0.6 µl) into electrophysiologically identified SmI digital loci, or visualized sulci. Survival times were 24-48 hrs. Sections were cut sagittally or transversely at 50 µm, reacted with TMB or DAB and counterstained with neutral red (TMB) or cresyl violet (DAB). Sections were examined under both bright-field and dark-field illumination for appearance and organization of retrogradely labelled VB cells in relation to thalamic architecture.

Focal injections of a gyral crown resulted in circumscribed labelling within the appropriate digital subnucleus of VB, while multiple injections encompassing an entire digital subgyrus resulted in labelling of the entire crescent-shaped subnucleus of VB. Injections of sulcal fundi led to more widespread but sparser labelling than did comparable injections of gyral crowns. Finally, injections of the central sulcus homologue did not result in preferential labelling of a separate, more rostrally situated subnucleus of VB (corresponding to VPLo of monkey; Horne and Tracey, 1979).

These results are in agreement with expectations based on prior electrophysiological studies of raccoon VB and SmI. There exists in the racoon a system of discrete, well-organized projections from VB hand subnuclei to SmI hand subgyri, with a preferential projection to gyral crowns, the regions of representation of the glabrous surfaces of the hand. (Supported in part by research grant NS-13418, USPHS.)

244.4 EFFECTS OF MEDIAN NERVE CRUSH AND REGENERATION ON THE ORGANIZATION OF THE HAND REPRESENTATION IN AREA 3B OF MONKEYS. J.T. Wall, D. J. Felleman, and J. H. Kaas. Depts. of Psychology & Anatomy, Vanderbilt University, Nashville, TN 37240.

Previous mapping studies of Area 3b of monkeys demonstrate that the somatotopic organization of the hand region undergoes major alterations when median nerve afferents from the hand are transected and when these afferents subsequently regenerate after transection. This study describes how the organization of the hand region is affected by crush and regeneration of the median nerve and compares the consequences of crush and transection injuries. The hand representation in Area 3b was defined by mapping receptive fields from multiunit responses to light tactile stimuli in 3 ketamine anesthetized owl monkeys with wrist level crush of the median nerve 32-170 days before mapping. Two of these monkeys were also mapped before crush to allow analysis of how receptive fields at identifiable (with respect to vasculature) cortical sites varied over time. In a monkey mapped 32 days after crush, the area of cortex representing median nerve inputs was smaller than normal and only representations of the palmar pads were apparent. Thus, at this relatively short regeneration time, the median nerve had reestablished connections with the palm but had not reinnervated the digits. Cortex normally representing digital skin innervated by the median nerve was activated instead from hand regions innervated by other nerves or was unresponsive. Similar changes in digital representations appear after transection and probably result from the crush injury. After longer regeneration times, all deafferented skin regions were reinnervated and the cortical area representing these inputs appeared normal in overall size and location. The sizes, locations, and topographic relationships of the representations of individual digits and pads contained in this area were also similar to normal. A comparison of these representations in repeatedly mapped monkeys indicates that although the pre- and post-crush maps are not identical, the observed differences can be relatively minor. For example, an analysis of the receptive fields at 52 locations in the hand region of a monkey studied 142 days after crush revealed that 79% of these sites had fields which overlapped the fields defined before crush. The small differences in pre- and post-crush maps were not entirely restricted to cortex receiving median nerve inputs and thus reflect possible variability in the representations of normal and regenerated inputs as well as errors in the remapping procedures. The reestablishment of an organized representation of the hand after crush related regeneration differs markedly from the disorganized reestablishment of connections observed when regeneration follows transection and may underly the better functional recovery usually reported after crush injuries.

(Supported by NIH grant NS 16466.)

244.5 SPATIAL FACILITATION AND OCCLUSION IN ASCENDING PATHWAYS FROM THE FORELIMB TO CAT SOMATOSENSORY CORTEX. D.D. Herman*, R.H. Kang* and P. Zarzecki. Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Individual neurons of cortical area 3a are known to receive convergent inputs from both muscle and cutaneous forelimb afferents. The purpose of the present study was to determine whether this convergence involves a sharing of neurons in ascending pathways projecting to area 3a. Synaptic potentials evoked by electrical stimulation of forelimb nerves were recorded from neurons of area 3a in Nembutal anesthetized cats. Incoming thalamocortical volleys evoked by each nerve were recorded from the cortical surface in parallel with postsynaptic potentials (PSPs). Four forelimb nerves were tested: two deep nerves (deep radial and palmar branch of the ulnar), and two skin nerves (superficial radial and dorsal cutaneous branch of the ulnar).

EPSPs were evoked from more than one forelimb nerve in almost all impaled neurons. Convergence of inputs from deep and skin nerves and from different regions of the limb was usual. For each neuron, tests for spatial facilitation and occlusion among convergent inputs were performed using minimal and maximal EPSPs respectively. Single pulse stimuli of the different nerves (1 Hz) were timed to synchronize incoming thalamocortical volleys. Two kinds of observations support the existence of common interneurons in the pathways from separate afferent sources. Weak stimulation of pairs of nerves evoked EPSPs larger than the sum of minimal EPSPs from individual nerves, due to facilitation of synaptic transmission through the afferent pathway. This spatial facilitation was observed between different regions of the limb ("topographic facilitation") and between skin and deep afferents ("cross-modality facilitation"). Near maximal EPSPs evoked from different parts of the limb were occluded, as were those evoked from deep and skin afferents. It is clear that convergence occurs prior to the impaled neurons and that some neurons are integrating inputs from several regions of the limb and from different modalities of afferents. This implies that the effectiveness of a given peripheral stimulus would be modifiable by stimuli of other modalities. Further analysis of the time course of the spatial facilitation and the magnitude of the occlusion may allow prediction of the site(s) of convergence and the degree of sharing along the ascending pathways.

(Supported by the MRC of Canada.)

244.6 TOPOGRAPHY, CYTOARCHITECTURE AND CONNECTIONS OF THE SOMATIC SENSORY AREAS OF THE ANTERIOR ECTOSYLVIAN GYRUS IN CATS. H. Burton and M. Kopf*. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110

The second somatic sensory area (SII) is found in the region of the anterior ectosylvian gyrus (AEG) in the cat. It was studied with multiunit recording techniques in anesthetized animals. Separate representations were noted for each of the forelimb (FL) digits and for a more posteriorly placed hindlimb (HL) toe zone in SII. Each of the FL digit zones occupied an anterior to posterior strip across the AEG with digit 5 medial and digit 1 furthest lateral. Respective proximal limb regions surrounded distal limb zones on nearly all sides. Another somatic area was located medial to the SII digit and FL zones and largely along the lateral bank of the anterior suprasylvian sulcus. It consisted of a rostral distal FL zone and a posterior distal HL representation. Both of these somatic regions were considered components of SII because of similar connections and related parakoniocortex cytoarchitecture, but the medial region appears more granular. Lateral to SII yet another somatic sensory area was found that was organized into rostral FL and caudal HL zones. This area lies buried along the upper bank of the middle extent of the AE sulcus and has previously been named SIV (Clemo and Stein, '82, Brain Res.). It has a more homotypical cytoarchitecture with an especially prominent continuous row of large layer V pyramids.

Injections of ^{3}H amino acids or HRP-WGA conjugate into each of these somatic sensory areas have shown that medial and lateral parts of SII connect reciprocally, substantially and topographically to area 4 and component parts of SI as previously shown, but additionally send small projections to the granular insula (Ig), area 43 of Hassler and Muhs-Clements ('64), area 5a, and possibly SIV. SII maintains reciprocal thalamic connections with VPL, CL and a small component of Po that lies dorsal to VP. Posterior parts of Po only receive corticothalamic projections from SII. In contrast, SIV projects to areas 6, 5, Ig, SSF of Rose ('49), area 35 and possibly SII. Dense reciprocal connections exist between Po and SIV. The deep layers of the superior colliculus and adjacent portions of the ventrolateral PAG receive a substantial projection from SIV but not SII.

Supported by NS09809.

244.7 TOPOGRAPHIC ORGANIZATION AND INTERHEMISPHERIC CONNECTIVITY OF CYTOARCHITECTURAL AREA S-II AND SURROUNDING CORTICAL FIELDS. O. Favorov, S. Juliano, B. Whitsel & C. Metz. Dept. Physiol. & Dental Res. Cntr., Univ. of NC, Chapel Hill, NC.

Two kinds of surface projection maps have been prepared for cerebral cortical cytoarchitectural area S-II and the fields which border it in Macaca fascicularis monkeys. The first demonstrates the position of ^{14}C-2deoxyglucose (2DG) metabolic labeling in unanesthetized animals subjected to controlled somatic stimuli delivered to different body regions. The second type of map demonstrates the position of neurons retrogradely labeled following horseradish peroxidase (HRP) injections in the postcentral gyrus (S-I) of the opposite hemisphere. The method of 2DG metabolic mapping for the somatic sensory nervous system has been described in detail (Juliano, et al., J. Neurophysiol., 1981); tetramethylbenzidine (Mesulam, J. Histochem. Cytochem., 1978) was used as the substrate to demonstrate neurons containing HRP in vibratome-cut sections of 50-100 um thickness. HRP injections which involved all or most of the contralateral postcentral gyrus labeled large numbers of neurons distributed over an extensive sector of the medial wall of the lateral fissure (LF) and insula. Labeled neurons were found within areas S-II, 7b, Ri and Ig. The most prominent feature of the summarizing projection maps is the presence of two large regions within cytoarchitectural area S-II which lack labeled neurons. These regions, in turn, are surrounded by territories (also within cytoarchitectural area S-II) containing such neurons. The latter territories can be subdivided further into regions distinguished on the basis of the density of labeled neurons. The patterns of 2DG labeling in area S-II and surrounding fields have been described previously (Juliano, et al., Neurosci. Abs. 7, 1981). Fractionation of the medial wall of the LF and the adjacent insula on the basis of callosal connections is virtually identical to that obtained with 2DG labeling. The two acallosal regions coincide with the distal limb representations, and the surrounding callosally connected regions correspond to the proximal and axial body representations. The plan of cortical organization revealed in the present study is similar to the "core-border" organization suggested for S-I in receptive field (RF) mapping experiments conducted in the absence of general anesthesia (McKenna, et al., J. Neurophysiol., 1982). The interpretation of the boundaries identified connectionally and in the metabolic mapping experiments appears consistent with those identified in single unit RF mapping studies. Supported by grants NS 10865 and DE 02668.

244.8 VARIATION IN RECEPTIVE FIELD SIZE ACROSS LAYER IV IN RAT BARRELFIELD CORTEX. J.L. Uhr, J.K. Chapin, and D.J. Woodward. Dept. of Cell Biology, U. Tex. Hlth. Sci. Ctr., Dallas, TX 75235.

The representation of the mystacial vibrissae in the rat primary somatosensory cortex consists of a matrix of about 30 adjacent "barrels", presumed to each represent a single whisker. In this cortical region the barrels consist of zones containing dense focal aggregates of layer IV granule cells, surrounded by less granular zones, or "septa." The purpose of this study was to determine if the receptive fields (RF's) of single neurons in layer IV exhibit sharp quantal transitions, or more gradual changes, as one crosses barrel boundaries in the course of multiple microelectrode penetrations in a line traversing barrelfield cortex (100 µm spacing). Computer generated post-stimulus time histograms were used to quantitate the responses of each of these layer IV neurons to standardized vibratory stimulation (single sinusoidal whisker displacements of 33 msec duration and 0.4 mm amplitude) of each of the whiskers comprising the neuron's RF.

Histograms from different units were compared by subtracting the background discharge rate from the discharge rate during the excitatory response epoch (7-30 msec from stimulus onset). These values were then normalized by plotting the measured response intensity of each whisker on a scale in which each one was expressed as a percentage of that of the whisker exhibiting the strongest response. Neurons responding to just a single whisker were found only within 100-200 µm areas near the centers of their corresponding barrels. As a corollary to this finding, the area of cortex responding to a particular whisker was found to be larger (extending up to 1 mm) than the diameter of a barrel (300-500 µm). Responses to stimulation of a single whisker typically exhibited a gradual decrease with increasing distance from the center of its corresponding barrel, and the response elicited from the whisker corresponding to the neighboring barrel increased gradually. However, this relatively continuous grading of RF's from one barrel to the next was somewhat broken in the septa between the barrels where the largest RF's were found. Neurons there often responded to stimulation of non-adjacent whiskers.

In summary, neurons in barrel centers responded only to a single whisker. Towards the barrel periphery neurons responded to that whisker and also to neighboring whiskers. Large RF's encompassing relatively distant whiskers were found in barrel septa. These large RF's may provide a physiological correlate to anatomical findings in this laboratory demonstrating that cortico-cortical axons from other whisker areas in the barrelfield terminate almost exclusively in the barrel septa.

This work was supported by grants NS18041 and AA0390.

244.9 QUANTITATIVE STUDIES OF THALAMOCORTICAL SYNAPSES WITH LABELED PYRAMIDAL CELLS IN MOUSE SmI CORTEX. Edward L. White, Steven M. Hersch and Cary R. Belford, Dept. of Anatomy, Boston U. Sch. of Medicine, Boston, MA. 02118.

Lesions of the nucleus ventralis posterior pars lateralis thalami(VB) were used to label thalamocortical (TC) axon terminals in layer IV of PMBSF cortex (SmI) in adult, male CD/1 mice. The degeneration of these TC axon terminals occurs over a very narrow time course such that at any postlesion survival time, all affected terminals in layer IV are at the same stage of degeneration; thus, their synapses can be meaningfully quantified. Pyramidal cells in PMBSF cortex were labeled by the retrograde transport of horseradish peroxidase injected into a) ipsilateral MsI cortex, b) the ipsilateral striatum or c) into the ipsilateral VB. Each injection resulted in numerous well-labeled cell bodies whose depths within PMBSF cortex depended on the site of injection: somata of cortico-cortical projection cells occurred primarily in layers II/III and V, corticostriatal somata occurred in uppermost layer V, whereas corticothalamic somata occurred in lower layer V and in upper layer VI. Examination of long, unbroken series of coronal thin sections through the layer IV portions of labeled dendrites showed the proportion of TC synapses formed by each type of dendrite to occur within a characteristic range. Although most dendrites passed through fields containing comparable concentrations of TC synapses, apical dendrites of corticostriatal cells formed 0.3-0.9% of their synapses in layer IV with TC axon terminals vs. 7-20% for the apical dendrites of corticothalamic cells. Basal dendrites of superficial cortico-cortical cells formed 1-7% of their synapses with TC axon terminals; apical dendrites of deep cortico-cortical pyramids formed essentially no TC synapses in layer IV. These latter dendrites were the only ones which consistently passed through regions of the PMBSF where TC axon terminals are rare (i.e. barrel sides and septa); all other dendrites examined in these studies traversed neuropil in barrel hollows where about 20% of all synapses were formed by degenerating TC axon terminals. These data indicate that specific cell types form characteristic proportions of TC synapses and that the proportion of TC synapses formed is not directly proportional to the concentration of TC axon terminals in the surrounding neuropil. In addition, these results suggest that apical dendrites belonging to certain types of cells possess specific spatial relationships with barrels in the PMBSF.

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244.10 CORTICO-CORTICAL CONNECTIONS BETWEEN PHYSIOLOGICALLY AND HISTOLOGICALLY DEFINED ZONES IN THE RAT SI AND MI CORTICES John K. Chapin and Donald J. Woodward, Univ. of Texas Health Science Center, Dallas, Texas, 75235.

The aim of these studies was to generate a proposal for assigning designations to the various subdivisions of the rat primary somatosensory (SI) cortex. In rodents, the SI cortex is observed as a discontinuous ring of discrete "granular zones", ie. discrete cortical regions containing dense aggregates of layer IV granule cells, each representing a different body part. We propose here that these be considered collectively as area 3b. Receptive fields (RF's) of units recorded here are small, almost exclusively cutaneous, and highly resistant to anesthesia. Less granular cortical areas, ("peri-granular zones", tentatively designated as area 1) are found just surrounding the granular zones. RF's in these areas are mainly cutaneous, but less discrete than in the granular zones and less resistant to anesthesia. The transitional cortical areas (3a) found just rostral to the forepaw and hindpaw granular zones contain neurons which respond to muscle stretch even in anesthetized animals, whereas in the more rostral MI cortex, neurons are not sensory responsive under anesthetic conditions. An oval shaped "central dysgranular zone" (area 2) is found near the center of the SI cortex. Neurons in this area are unresponsive to any sensory stimuli in deeply anesthetized animals but exhibit both cutaneous and muscle/joint RF's in awake animals. A small "posterior parietal cortex", (area 5), is located just caudal to the SI cortex.

Horseradish peroxidase and autoradiographic techniques were used to map the ipsilateral connections between these areas. Cells in granular zones were observed to project to adjacent peri-granular zones, and these in turn projected to a distinct area within the central dysgranular zone (area 2). Area 3a also projected strongly to area 2. Area 2 itself projected further caudally to the parietal cortex. Topographically distinct areas in the MI exhibited reciprocal connections with corresponding somatotopic areas in all the above zones in SI. In general, regions that received strong projections from MI were areas which in turn projected strongly back to MI. The relative strength of these reciprocal connections with MI may be ranked in approximately the following order: 2,1,3a,5,3b.

Overall, despite some important differences, the physiological and anatomical characteristics of the cortical subdivisions tentatively defined here as areas 3a, 3b, 1, 2, and 5, correspond rather well with those of similarly named cortical strips in the primate. Supported by grants NS-18041, AA-0390, and the Biological Humanics Foundation.

244.11 THE ORIGIN OF THE SOMATOSENSORY CORTICOTECTAL PATHWAY IN CAT. Barry E. Stein, Robert F. Spencer and Stephen B. Edwards. Dept. of Physiol. and Anat., Med. Coll. of Va., Richmond, VA 23298 and Dept. Anat. Univ. Va., Charlottesville, VA 22901.

One assumption that has evolved from information regarding the visual corticotectal pathway is that each sensory modality represented in the superior colliculus (SC) receives a descending input from its primary cortical representation. Thus, it was surprising to find that whereas cooling visual cortex in cat depressed visual cells in the SC, cooling somatic cortex (SI-SIII) had no influence on somatic SC cells (Stein, B.E., J. Neurophysiol., 41:55, 1978). We therefore sought to determine if there was a somatic projection from cortex to the SC in cat. Restricted injections of tritiated leucine were made in separate animals in physiologically-identified areas of SI, SII and the adjacent anterior ectosylvian sulcus (AES). The AES was included because it has recently been shown to contain a fourth complete map (SIV) of the body surface (Clemo, H.R. and Stein, B.E., Brain Res., 235:162, 1972). Surprisingly, the autoradiographic terminal labelling did not exceed background levels in the SC after injections of SI and SII despite heavy labelling in the ventrobasal complex. However, terminal labelling in the SC was apparent after injections of leucine into the AES.

In subsequent experiments retrograde transport of HRP confirmed the projection from the AES to the SC and showed only a sparse projection from SI-SIII. The projection from the AES originated from medium-diameter pyramidal cells in lamina 5 of the inferior and superior banks of the sulcus.

Injections of HRP into the AES resulted in anterograde terminal labelling in the intermediate and deep laminae of the SC, extending into the dorsolateral periaqueductal gray. The projection was primarily, though not exclusively, ipsilateral. Confinement of the spread of HRP within the region of the AES was indicated by both anterograde and retrograde labelling in the posterior thalamic nuclear group, with little, if any, labelling in the ventrobasal complex. In a separate, but related series of studies, microstimulation of physiologically-identified regions of the AES produced short latency activation of somatic SC cells whose receptive fields closely matched those at the stimulation site in cortex. Stimulation of SI and SII had little effect on SC cells (Clemo, H.R. and Stein, B.E., Neurosci. Abstr. 7, 758, 1981). These observations support the contention that the AES (SIV), and not traditionally defined somatosensory cortex, is the major source of descending somatic input to the Cat SC.

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245.1 ANALGESIC-LIKE EFFECTS OF DIMETHYL SULFOXIDE ON C FIBER ACTIVITY IN THE CAT: EVIDENCE FOR MORE THAN ONE MECHANISM OF ACTION. K. H. Reid, M. S. Evans* and L. R. Sharp, Jr.*. Dept. Physiology and Biophysics, Univ. of Louisville, Louisville, KY 40292.

Dimethyl sulfoxide is a chemical solvent used in veterinary medicine to treat bruises, sprains and strains. It has no well-documented curative effect, but is used for analgesia and vasodilation. Both these functions are mediated mainly by unmyelinated (C) fibers; we have evaluated some effects of dimethyl sulfoxide on these fibers in the sural nerve of the barbiturate-anesthesized cat. Concentrations of 1% to 40% in Hanks Balanced Salt Solution were applied to the exposed nerve for periods of 3 minutes to 6 hours. The nerve was first carefully dissected free from the underlying muscle, with both ends attached and its major blood supply intact, and sustained in water-saturated air at 37 C. It was then inserted through a slit in a glass tube of volume 0.4 ml, which was then filled with the test solution. Leakage was minimized by using an infusion pump to provide a slow withdrawal of fluid from the tube during recording. The sciatic nerve was tied off proximal to the sural branch, and bipolar stimulation, using pulses of 0.5 msec duration applied at 3/sec, was used to test conduction. Bipolar recording electrodes distal to the bath were used to record the responses of the nerve to the stimulus and to the rubbing of hairs in its peripheral receptive field. Groups of 16 responses were averaged and plotted on a strip chart for examination. Sample single responses were recorded from an oscilloscope display using a Polaroid camera.

At concentrations above 8% we observed 'fast block'. This was seen as a loss of the C fiber response within 30 minutes of drug application. The waveform diminished without substantial change in shape, and recovered 5-20 minutes after washing the nerve with the vehicle. At concentrations of 1% to 7%, we observed 'slow block'. This took much longer (2-3 hours) to develop, and was accompanied by an extensive distortion and fractionation of the waveform. This block was also reversible. Application of pure balanced salt solution for up to 9 hours did not induce block. In agreement with Becker et al (1969) we found that concentrations over 40% produced an irreversible block.

In veterinary practice topical dimethyl sulfoxide is normally used at concentrations of 50% to 90%, and produces effects within a few minutes (fast block). Systemic dimethyl sulfoxide is given in doses that produce tissue concentrations under 1%, and the time of greatest effect is 1-3 hours post-injection (slow block). As dimethyl sulfoxide has numerous modes of interaction with biological processes, other modes of block may also exist. The effects demonstrated in this study appear adequate to account for some analgesic and vasodilatory actions seen when this agent is used in veterinary practice.

245.2 CHARACTERISTICS OF PAIN CELLS ARE EXPRESSED BY SENSORY NEURONS IN CULTURE. P.I. Baccaglini and P G Hogan*. Neurobiology, Harvard Med. Sch., Boston, MA 02115

The existence of a class of primary sensory neurons (nociceptors) specifically activated by painful stimulation has been clearly established in mammals. Nociceptive sensory endings are sensitive to compounds produced locally during inflammation and painful pathological states. Among these compounds are bradykinin, prostaglandins and amines. Since it has been difficult to elucidate in vivo the mechanisms of action of these compounds, an alternative approach is to study pain neurons in cell culture.

Sensory neurons were dissociated from dorsal root ganglia (DRG) and trigeminal ganglion (TG) of newborn rats, and grown in culture Drugs were applied from a micropipette (tip diameter 10-25 um) by brief pressure pulses causing the ejection of "puffs" of solution to a restricted region of the neuron.

Many neurons in culture expressed characteristics of pain sensory cells. The characteristics tested were sensitivity to low concentrations of capsaicin (CAP) and bradykinin (BK), sensitization with prostaglandin E_2 (PGE_2), and presence of substance P-like immunoreactivity (SP-LI).

CAP has been reported to be a selective stimulus for pain sensory neurons in vivo. Many neurons in culture (80% DRG; 69% TG) were excited by low concentrations (10^{-9}-10^{-7}M) of CAP, while others did not respond even at higher concentration (10^{-5}M). These excitatory responses (action potentials and depolarization) outlasted the stimulus for several seconds and were not blocked by low Ca^{++}-high Mg^{++} solutions suggesting that most sensitive neurons respond directly to CAP.

BK (10^{-10}-10^{-7}M) also excited a majority of the neurons (72%DRG; 89% TG). Typically the response was a slow depolarization (2-10mV amplitude; 30-150 s) and trains of action potentials. Most neurons showed reduced responses when BK was applied every minute.

PGE_2 (10^{-9}-10^{-7}M) enhanced the response to all the algesics we tested: CAP, BK, and K^+. When PGE_2 was present in the perfusion medium the number of action potentials in response to a given algesic was increased 1.2 to 6.6-fold.

SP-LI was present in many neurons of both DRG and TG cultures. With the PAP technique, 43% of the neurons in TG cultures stained with an antiserum to SP; the rest were unstained.

The results indicate that: 1) many sensory neurons in DRG and TG cultures express properties of differentiated pain neurons; 2) sensitivity to BK and sensitization with PGE_2 to algesics are properties of the neurons themselves in the absense of any target tissue. (Supported by NS11576, NS03273, NS02253; Am. Heart Assoc. 78-964).

245.3 HUMAN NOCICEPTOR SENSITIVITY TO INCREMENTAL THERMAL STIMULI: A COMPARISON WITH PSYCHOPHYSICAL DETECTION AND PAIN RATING THRESHOLDS AND SCALING. C.J.Robinsor R.H.LaMotte, H.E.Torebjork* RER&D Center, Hines VA Hospital, Hines, IL 60141 and Dept of Anesthesiology, Yale Univ, New Haven, CT 06510

The human capacity to detect and discriminate between small increments in temperature is better when the increments are superimposed on painful, as opposed to non-painful adaptation (base) temperatures (Robinson et al '80 Soc for Neurosci Abstr). To determine a possible peripheral neural substrate for this ability, we have percutaneously recorded activity in single C-fiber mechanoheat nociceptor afferents(CMH's) in peroneal nerve of awake humans, who were simultaneously judging pain magnitude.

Seven human subjects, each of whom gave informed consent to an approved protocol, made continuous ratings of the magnitude of pain sensation, if any, evoked by base temperatures of either 38 or 48°C upon which temperature increments, each of 5 seconds duration,were superimposed every 30 seconds. Increments for bases of 38 and 48°C were respectively 1.0 to 13.0°C and 0.1 to 1.3°C.

Measures of responses in 12 CMH's with receptive fields on the distal leg or dorsum of the foot were: the total number of impulses evoked by each increment, the net evoked response (total - background), and instantaneous discharge frequency. The nociceptors had no spontaneous activity on the non-painful base of 38°C, and response thresholds were 3°C(10 nociceptors) or 5°C(2 nociceptors). In contrast, pain thresholds were between 3 and 11°C. These thresholds were an order of magnitude higher than detection thresholds measured on 38°C (op. cit.).

Response thresholds of the 6 CMH's that were subjected to a 48°C base ranged from 0.2 to 0.5°C. Incremental pain thresholds on a 48°C base ranged from 0.1 to 0.5°C. These thresholds were comparable to detection thresholds (0.1 to 0.3°C) measured previously on a 47°C base (op.cit.).

The peak magnitude ratings of pain evoked by increments on the 48°C base generally increased monotonically with increasing size of the increments. The total and net number of impulses evoked in each CMH were non-monotonic, but generally increasing, with increasing size of the increments. After the larger increments, there was a marked transient reduction in each nociceptor's response to the 48°C base and a similar decrease in pain rating.

Incremental pain sensitivity was not altered by a compression block of activity in myelinated afferents that eliminated the sense of cool and touch. Thus, activity in unmyelinated fibers alone could account for the sensitivity to incremental thermal stimuli that were superimposed on a painful base temperature. Further, it is likely that CMH nociceptors could provide the peripheral information necessary to detect and to make magnitude judgments of pain elicited by these stimuli.

245.4 NEURAL MECHANISMS OF HYPERALGESIA: EFFECTS OF PARTIAL INJURY TO THE RECEPTIVE FIELD OF NOCICEPTIVE AFFERENTS. J.N. Campbell (1), R.A. Meyer (1,3), S.N. Raja (2), R. Burke* (1) and J.J. Aryanpur* (1). Dept. of Neurosurgery (1), Dept. of Anesthesiology and Critical Care Medicine (2), and Applied Physics Lab. (3), Johns Hopkins Univ., Baltimore, MD 21205.

We previously demonstrated that a 53°C 30s burn applied to a 7.5 mm diameter spot on the glabrous skin of the hand in humans caused thermal hyperalgesia. When the burn was applied to the center of the receptive field of A-fiber (AMHs) and C-fiber (CMHs) nociceptive afferents sensitive to mechanical and heat stimuli in the monkey, the AMHs sensitized whereas the CMHs suppressed. We concluded that AMHs, not CMHs, code for the hyperalgesia following this injury. Others have shown that following less severe injuries to the glabrous skin, CMHs develop a lower heat threshold and thus might play a role in hyperalgesia resulting from mild injuries. It is likely that only part of the receptive field of several AMH and CMH receptors was stimulated in the human experiments. Since a partial receptive field burn might be equivalent to a mild injury and thus sensitize CMHs, we wished to determine the effects of a 53°C 30s burn applied only to part of the receptive field of glabrous CMHs and AMHs. The laser thermal stimulator was positioned such that the edge of the stimulus abutted the center of the receptive field, and thus less than half of the receptive field area was within the area of the stimulus. Six CMHs and 6 AMHs with receptive fields completely on glabrous skin were studied. A thermal test sequence (TTS) was used to determine the thermal responses 5 minutes before and ten minutes after the 53°C 30s burn. The TTS consisted of ten 3s stimuli with a 27s interstimulus interval. Following an initial 45°C stimulus, nine stimuli ranging from 41°-49°C in 1°C increments were presented in random order. The thermal threshold and total evoked response of the CMHs to the TTS did not change significantly following the burn. In contrast, the AMHs developed a significantly lower thermal threshold and higher total evoked response following the burn. Therefore a partial receptive field injury with a 53°C 30s heat stimulus did not sensitize CMHs, but sensitized AMHs. These data further support our previous conclusion that AMHs not CMHs code for hyperalgesia after a major injury to the glabrous skin. (Supported by N.I.H. grants #NS-00519 and NS-14447, D.O.D. contract #N00024-81-C5301, and the John J. Hopkins Fund)

245.5 GENERAL ANESTHETICS AFFECT THE RESPONSE OF PRIMARY NOCICEPTIVE AFFERENTS IN PRIMATES. S.N. Raja (2), R.A. Meyer (1,3), J.N. Campbell (1), and R. Burke* (1), Dept. of Neurosurgery (1), Anesthesiology and Critical Care Medicine (2), and Applied Physics Lab. (3), The Johns Hopkins Univ., Baltimore, MD 21205.

The response properties of unmyelinated nociceptive afferents, sensitive to both mechanical and heat stimuli (CMHs), were compared in primates anesthetized with either barbiturates, or halothane and nitrous oxide (N_2O). In an initial study, we compared the responses of 22 CMHs in primates under pentobarbital anesthesia with the responses of 17 CMHs in primates under a combination of halothane (0.8%) and N_2O (60%) anesthesia. A laser thermal stimulator was used to deliver a thermal test sequence (TTS), which consisted of ten 41-49°C 3s stimuli, to the receptive fields of the CMHs. Mechanical thresholds were determined with Von Frey hairs. The thermal threshold was significantly lower ($p<0.001$) in CMHs in primates under halothane/N_2O anesthesia as opposed to pentobarbital anesthesia. The mechanical threshold also was significantly decreased for CMHs that innervated glabrous skin under conditions of halothane/N_2O anesthesia, but this difference was not seen in hairy skin CMHs. The total evoked response to the TTS was also significantly greater under halothane/N_2O anesthesia ($p<0.001$). These results prompted four cross-over studies in which the responses of a CMH to the TTS were first recorded with the primate under halothane/N_2O anesthesia, then under an ultra-short-acting barbiturate (methohexital), and finally after returning to halothane/N_2O. The respirations were controlled in each experiment, and in one experiment the end-tidal CO_2 was monitored and maintained at the same level under each anesthetic condition. The mean total response to the TTS under barbiturate anesthesia was 55% $\pm$ 5% (+ S.E.M.) of that under halothane/N_2O anesthesia. In each case, the responsiveness increased once the halothane/N_2O was reinstituted. Thus, for both the population study and the cross-over study, the responsiveness of the CMHs was greater under halothane/N_2O than under barbiturate anesthesia. Preliminary results indicate that A-fiber nociceptive afferents also exhibit enhanced sensitivity under halothane/N_2O anesthesia. Whether the choice of anesthetic modifies the response of other types of primary afferents has yet to be determined. These data challenge the commonly held assumption that anesthetics do not significantly modify the response of primary afferents. (Supported by N.I.H. grants #NS-00519 and NS-14447, D.O.D. contract #N00024-81-C-3301, and the John J. Hopkins Fund)

245.6 NEURAL ACTIVITY ORIGINATING FROM A NEUROMA IN THE BABOON. R.A. Meyer (1,2), J.N. Campbell (1), S.N. Raja (1), S.E. MacKinnon* (3), R. Burke* (1), and A.L. Dellon* (1,3). School of Medicine (1) and Applied Physics Laboratory (2), The Johns Hopkins University and Union Memorial Hospital (3), Baltimore, MD 21205

It is clinically observed that neuromas of the superficial radial nerve at the wrist often result in chronic pain. This pain, which typically develops weeks to months after injury, may be spontaneous and is usually aggravated by mechanical pressure at or near the neuroma. In the baboon we determined that unmyelinated and myelinated nerve fibers from the superficial radial nerve in which a neuroma was produced exhibited both spontaneous activity and activity induced by mechanical pressure on the neuroma. Coupling of activity between nerve fibers at the neuroma (presumably due to ephaptic conduction) was also observed. In five baboons all branches of the superficial radial nerve at the wrist were crushed with a hemostat for 15 s and 2 cm of the nerve distal to the crush were removed. Within 1 month, a neuroma bulb could be palpated near the site of the crush. A teased fiber technique, in which the proximal end of fine strands of nerve fibers was severed, was used to record single fiber action potentials. Spontaneous activity and activity induced by mechanical pressure on the neuroma were recorded. An electrode was placed on the nerve between the recording site and the neuroma in order to determine conduction velocity. A second electrode was placed on the nerve proximal to the recording site and was used to assess "ephaptic" conduction at the neuroma. Four neuromas were studied at the age of 1 to 2 months. A total of 122 unmyelinated and 93 myelinated fibers were studied. Ten percent displayed spontaneous activity with rates ranging from 1 to 60 impulses per minute, and 22% responded to mechanical stimulation of the neuroma. No "ephaptic" conduction was observed. In two 4-month-old neuromas, spontaneous activity and mechanically induced activity were similar to that seen in the 1 to 2 month neuromas. Three instances of "ephaptic" conduction were observed as indicated by a synchronized response to stimulation with the proximal electrode. This response was eliminated by application of a local anesthetic (Procaine) to the neuroma. The observed spontaneous and mechanically induced activity in unmyelinated as well as myelinated fibers might account for the spontaneous and mechanically induced pain experienced by patients with neuromas. (Supported by N.I.H. grants #NS-00519 and NS-14447, D.O.D. contract #N00024-81-C-5301, and the John J. Hopkins Fund)

245.7 SEARCH FOR A SPECIFIC, ITCH-SIGNALING RECEPTOR. R.P. Tuckett, Department of Physiology, University of Utah, Med. Ctr., Salt Lake City, Utah 84108.

In an earlier report a pruritic substance (cowhage) was applied to the various receptor populations in cat hairy skin and only the polymodal nociceptor was found to respond (Tuckett, Neurosci Abstr 6:428, 1980). However, since the polymodal nociceptor also responds to stimuli that produce pain in man, it is difficult to envision how this single population could be signaling the two separate sensory modalities of itch and pain. The observation that people consistently felt itching when their hairy skin was stimulated electrically over a range of frequencies argues against the possibility that the polymodal population signals both itch and pain by generating different patterns of activity for each type of stimulus (Tuckett, Neurosci Abstr 7:531, 1981). Alternatively, it is possible that itch is signaled by a specific receptor. Since this hypothetical receptor would respond only, or predominantly to pruritic stimuli, it could have been missed in earlier studies which used mechanical and thermal stimuli to search for the receptive fields of neurons activated by electrical stimulation of the whole peripheral nerve. One way to search for a specific receptor would be to apply a pruritogen to the whole nerve field and isolate single units that have been activated. There are several problems with this approach, one being that the polymodal neurons, which comprise a significant portion of the unmyelinated afferent population, would also be activated adding to the difficulty of distinguishing an unknown receptor. In addition if such a receptor were recorded from, the problem of locating its receptive field would remain.

An alternative approach was based on the observation that electrical stimulation of hairy skin in man evokes pruritus (see above); and consequently, that the itch-signaling receptor is amoung those activated by electrocutaneous stimuli. In cat, filaments of the sural nerve were recorded from and an electrical stimulus passed over the skin, searching for slowly conducting fibers. The receptive fields of 86 unmyelinated and 10 myelinated neurons were isolated by the electrocutaneous search stimulus and subsequently characterized by their response to thermal and mechanical stimuli. All were found to belong to known receptor categories: myelinated nociceptor, thermal receptor, C-mechanoreceptor and polymodal nociceptor.

It was concluded that there is no evidence to support the possibility that itch is signaled by an unknown receptor population. Hence, it is likely that the polymodal nociceptor is involved.

245.8 A COMPARISON OF PRIMARY AFFERENT THERMORECEPTORS WITH MEDULLARY AND THALAMIC NEURONS IN THE CAT TRIGEMINAL SYSTEM. D.A. Poulos and H. Hirata. Dept. of Anatomy and Div. of Neurosurgery, Albany Medical College, Albany, N.Y. 12208.

The present study was done to compare the response characteristics of primary afferent trigeminal cold receptors with those of their central counterparts located in the spinal trigeminal nucleus and VPM of thalamus. Our purpose was to determine whether certain populations of central neurons whose behavior differed from that reported for primary afferents reflected central processing and convergence or input from previously unrecognized primary afferent fibers. More specifically, typical cold receptors (T units) give dynamic responses to sudden cooling, show rate decreases to warming, maintain static temperature dependent activity over a wide range of temperatures held constant, and are insensitive to mechanical forms of stimulation. In thalamus (Neurosci. Abst. 5:489, 1979) we found, in addition to T units, a group of neurons responsive to both thermal and mechanical forms of stimulation (T & M units). In medulla (Neurosci. Abst. 3:711, 1977), T & M units were not observed. However, we found, in addition to typical T units, T units that displayed 1) only temperature dependent static activity and 2) only dynamic responses to rapid cooling.

Primary afferent data were obtained by recording extracellular discharges from cells located in the trigeminal ganglion of cats anesthetized with Nembutal and/or urethane anesthesia. Peripheral receptive field identification, thermal stimuli, and stimulus paradigms were identical to those used in studies of central neurons. Many thermoreceptive neurons were identified and 22 were held electrophysiologically isolated for sufficient time to study their entire range of static and dynamic responsiveness. All of the primary afferent neurons were found to be typical T units. We conclude that 1) thalamic T & M units may represent a convergence of specific thermoreceptor and mechanoreceptor inputs and 2) the medullary static and dynamic neurons reflect a central processing of primary afferent information.

Supported by NIH Grant NS 11384.

245.9 FUNCTIONAL AND ANATOMICAL PROPERTIES OF NEURONS INNERVATING THE MAXILLARY CANINE TEETH OF CATS. T. Jones*, K. V. Anderson, N. F. Capra and Y. Khawaji*. Section of Craniofacial Biology, Univ. Mississippi Med. Center, Jackson, MS 39216.

While it is known that both myelinated (M) and unmyelinated (UM) axons innervate the teeth, their cells of origin and anatomic features remain the subject of debate. The present study was designed to characterize the morphological properties of primary afferent neurons that innervate the maxillary canine teeth (MCT) of cats. Special attention was focused on the sensory neurons whose cell bodies reside in the trigeminal ganglia (TG) and whose axons project to the teeth. TG neurons that innervate the MCT were tagged by placing a horseradish peroxidase (HRP) solution into the pulp chamber of MCT and processing TG tissue 24-48 hours later. Cells innervating the teeth exposed to HRP showed a clearly observable reaction product. The axonal characteristics of sensory nerves supplying the MCT were determined from tissue taken from the juxtaapical region of the teeth. The sympathetic motor nerves entering the neural pulp were eliminated by subjecting animals to a bilateral sympathectomy 30 days prior to other phases of the experiment. In all, 13 cats were used in these studies. The evaluation of TG cell soma features and of afferent axonal profiles was accomplished with a computer-based image analysis system.

To date, more than 2000 TG neurons innervating MCT have been studied. While most such neurons were located in the maxillary portion of the TG, some were found in mandibular or ophthalmic regions of the TG. Image analysis showed that most TG neurons that supply the MCT were basically circular structures with an average area of 1496 microns, an average perimeter of 164 microns and an average maximum diameter of 56 microns. The soma varied widely in their sizes and ranged from the smallest to the largest of TG neurons. An average of 354 TG neurons innervated ipsilateral MCT.

The properties of sensory tooth nerves were determined from photomontages constructed from electron micrographs. Each montage depicted the entire neural contribution to each tooth. On the average, 337 axon profiles were observed at the entry point to each maxillary canine tooth. Of these, 255 were M axons and 82 were UM axons. M profiles ranged from 0.75-10.0 microns in diameter and UM profiles from 0.2-3.0 microns in diameter.

It would appear that there is about a one-to-one ratio of neurons innervating MCT and sensory axons supplying each maxillary canine tooth. In addition, TG neurons that supply the MCT are relatively large and innervate the teeth primarily by way of M axons in the A-delta range. One wonders if the larger TG neurons project to MCT via M axons, while smaller TG neurons project via UM axons.

245.10 SENSORY RECEPTOR STRUCTURE IN PERIODONTAL LIGAMENT OF THE RAT. Margaret R. Byers and L.J.Laughran*. Anesthesiology, Biological Structure, and Center for Research in Oral Biology; University of Washington, Seattle, WA, 98195.

Sensory receptors in periodontal ligament are important for sensitivity of teeth to touch or displacement, for mastication reflexes, and for periodontal pain. Their nerve cell bodies are found in the trigeminal Gasserian ganglion or the mesencephalic nucleus. We have labeled the Gasserian nerve endings by rapid axonal transport of ^{3}H-protein in order to distinguish them from the mesencephalic endings, and to study their fine structure, their association with terminal Schwann cells, and their relationship to basal lamina and ligament collagen.

We found many labeled nerve endings near the base of the molar roots that were either free endings or special mechanoreceptors. The free endings were found near blood vessels and among ligament fibers: they contained occasional clusters of vesicles and mitochondria; they were separated from the ligament by a basal lamina; and they were partially covered by a Schwann cell.

The special mechanoreceptors were elaborate branched nerve endings that originated from myelinated axons located within a perineurial sheath. At nodes of Ranvier, unmyelinated branches left the sheathed axon, moved among the fibers of the avascular ligament, and gave off many terminal branches. The endings were surrounded by specialized terminal Schwann cells that were filled with numerous pinocytotic pits and vesicles. Beyond the Schwann cell, many asymmetric layers of basal lamina were often found. Each nerve ending had a central region filled with many mitochondria; it also gave off projections that passed between the Schwann cells and the multilayered basal lamina to end among the collagen fibers of the ligament. These projections were devoid of organelles except for fine filaments; in the axoplasm near their base, numerous clear vesicles were found as well as occasional dense core vesicles, multivesicular bodies, and profiles of smooth endoplasmic reticulum. The projections resemble proposed transducer sites of other partially or fully encapsulated mechanoreceptors (Andres and von During, Handb.Sens.Physiol., 2:3, 1973: Gottschaldt et al, J.Comp.Neurol. 205:219, 1982). The unlabeled endings in periodontal ligament, which included those of mesencephalic origin, had the same structure as the labeled Gasserian endings. No fully encapsulated endings were found. The special Gasserian mechanoreceptors are positioned optimally to detect compression or stretch of the ligament collagen bundles; their structure suggests that they participate in the tactile and proprioceptive sensitivity of teeth as well as in mastication reflexes. (Supported by grants DE05159, DE00099, DE02600.)

245.11 A QUANTITATIVE INVESTIGATION OF THE AFFERENT INNERVATION OF SINUS HAIR (SH) FOLLICLES IN THE CAT. K.-M. Gottschaldt and B. Kyau*. Dept. Neurobiol., MPI Biophys. Chemistry, 34 Göttingen, FR Germany.

The innervation of SH follicles was studied quantitatively using light- and electron microscopical techniques. The 49 maxillary SH follicles on one side of the face received altogether 4500 myelinated axons. The number of nerve fibres entering a single SH follicle varied between 30 and 207 and increased with the size of the follicle. As the fibres enter the follicle their diameters vary between 1 and 10 µm averaging between 3.8 and 4.6 µm in different follicles. No relationship was found between mean axon diameter and size of a SH follicle or the number of axons innervating it. While ascending within the trabeculae of the cavernous sinus the axons branch, on average 1.5 times, and decrease in diameter so that further up in the follicle many more axons can be found than are entering it. With the myelinated axons, about four times as many unmyelinated axons enter the follicle. Most of these are very thin (<.5 µm in diameter) and appear to terminate in the trabeculae of the cavernous sinus. About one third of the unmyelinated axons terminate in the inner hair follicle near the glassy membrane where also the mechanoreceptive nerve endings of myelinated axons are located.

The small myelinated axons (diameter 2 µm or less) amount to about 20% of the myelinated axons and terminate near or at the vascularized hair papilla but not in mechanoreceptive nerve endings. The latter are located in the middle third of the hair follicle and five morphological types can be distinguished: 1) Lamellated (Golgi-Mazzoni) corpuscles and 2) branched lanceolate nerve endings of the Ruffini ending type occur in the inner hair follicle around the lower end of the hair shaft, not more than 10 of each type in a medium-sized follicle. At the level of of the ringwulst there are 3) large straight lanceolate nerve endings which are attached on one side to the glassy membrane and on the other side to the connective tissue of the inner hair follicle. At the level of the external root enlargement the latter receptor type is replaced by 4) a smaller type of lanceolate nerve ending which is branched and attached only to the glassy membrane. This newly identified receptor type resembles morphologically the palisade endings in ordinary hair follicles and may give rise to the electrophysiologically recorded rapidly adapting velocity responses. Finally, there are 5) thousands of Merkel cells in the external root sheath of which, however, only about 50% are associated with a sensory nerve terminal. The available detailed knowledge about number, size and termination sites of the myelinated axons and of the number and location of the different receptor types now enables a more accurate correlation between physiological response types in afferent fibres and morphological receptor structures within the SH follicle. Supported by DFG-SFB 33.

245.12 A STUDY OF CODING IN PRIMARY AFFERENTS IN THE RACCOON: THE NEURAL REPRESENTATION OF MECHANICAL STIMULI VARYING IN LOCATION AND INTENSITY. R.H. Ray and G.S. Doetsch. Dept. Physiol., East Carolina Univ., Greenville, NC 27834 and Depts. Surgery (Neurosurg.) and Physiol., Medical College of Georgia, Augusta, GA 30912.

The across-fiber pattern model was used to examine coding of stimulus location and intensity in primary afferent nerve fibers. According to the model (see Doetsch and Erickson, 1978; Ray and Doetsch, 1978), information about the specific location of a stimulus is coded in the ratios of activity across all responding fibers (across-fiber patterns or AFPs) and intensity is coded in the total activity of the responding fiber population. Data were obtained from fibers with receptive fields which included at least 1 of 6 standardized test points. Data from 129 median and 61 tibial nerve fibers showed the following: (1) Threshold. Distributions of threshold as determined with calibrated nylon filaments were not significantly different for the median or tibial nerve samples or for different receptor classes (RA, MSA or VSA). A distal to proximal increase in threshold was observed, suggesting a gradient of sensitivity across the paws. (2) Receptive field (RF) area. Threshold RF areas did not vary significantly across any one paw or between paws. Suprathreshold RFs showed a distal to proximal increase in area on each paw and were quite large relative to expected acuity, however RFs on digit 1 of the forepaw did not differ from those on digit 1 of the hindpaw, suggesting that RF area alone cannot account for presumed differences in acuity. (3) Innervation density (ID). A sharp distal to proximal decrease in ID across each paw was demonstrated by comparing the integrated voltages of compound action potentials evoked by constant current stimuli delivered to each test point. A forepaw to hindpaw ID ratio of 2.5:1 was estimated from counts of myelinated fibers and measurements of skin areas innervated by the median and tibial nerves. These findings are consistent with the AFP model since increasing ID increases the differential neural activity of the fiber population that underlies discriminability. (4) Firing rates. Spike discharge as well as adaptation rate was shown to depend upon both stimulus location and intensity. A single fiber therefore cannot unambiguously encode either stimulus parameter.

Reconstruction of AFPs for different stimulus locations and intensities demonstrated unambiguous representation of these stimulus parameters. Consistent with expectations based upon human psychophysics, AFPs for stimuli delivered to points on distal glabrous skin showed greater sensitivity to changes in stimulus location and intensity than AFPs produced by stimuli delivered proximally. Furthermore, AFPs produced by stimuli delivered to the forepaw showed greater sensitivity to stimulus changes than AFPs representing stimuli delivered to comparable hindpaw skin regions. (Supported by GRS Grant, NIH, 5S01-RR05365-16)

245.13 THE INFLUENCE OF RATE OF STIMULUS INDENTATION ON THRESHOLD AND SUPRATHRESHOLD TACTILE SENSATIONS. D. R. Kenshalo, Sr., J.D. Greenspan and R. Henderson*. Psychology Department, Florida State University, Tallahassee, FL 32306

Absolute tactile thresholds, measured in terms of skin indentation depth, were determined as functions of the rate of indentation. Rates between 0.2 and 10 mm/sec had little effect on the absolute thresholds. Slower rates resulted in increased absolute thresholds.

Estimates of the magnitude of tactile sensations as a function of depth of indentation, with rate as a parameter, were also made. All rates had consistent influences on the judged intensity of the tactile sensations. The fastest rate, for a given depth of indentation, produced the most intense sensation, the slowest, the least intense sensation. Intermediate rates were ordered between these extremes.

The magnitude estimates of indentation depth could be described by a power function. At the slowest rate, 0.1 mm/sec, the exponent of the function was 1.39. At higher rates of indentation, 0.4, 1.0, and 10 mm/sec, two exponents were required to fit the entire function. The exponents of the power functions were between 0.4 and 0.6 for indentation depths up to about 1 mm and between 1.1 and 1.6 for deeper depths of indentation.

A neural basis is proposed to account for these results.

245.14 MODULATION BY SYMPATHETIC OUTFLOW OF ACTIVITY IN SKIN AFFERENTS IN CONSCIOUS MAN. R.G. Hallin* and Z. Wiesenfeld-Hallin (SPON: S. Grillner), Department of Clinical Neurophysiology, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

Results from animal experiments indicate that the sympathetic outflow can modulate the responsiveness of cutaneous afferents. Peripheral nerve lesions in rats and mice can lead to abnormal connections between sympathetic efferents and sprouts of afferent fibers. Furthermore, many clinical states are accompanied by symptoms of sympathetic dysfunction and some painful conditions are relieved by sympathetic block or sympathectomy.

Until recently it has not been possible to study the possible link between cutaneous afferent and sympathetic efferent activity in conscious man. We have developed a standardized concentric electrode for percutaneous recording of A and C fiber units in humans (Acta Physiol. Scand., 1981, 113). With this electrode activity from single afferent skin fibers is regularly recorded simultaneously as signals from sympathetic fibers can be discriminated. In recordings from the median or peroneal nerves in healthy subjects we studied particularly how units innervating Pacinian corpuscles were modulated by changes in the sympathetic outflow.

During the experiments the subjects were comfortably reclining and quietly awake. The cutaneous sympathetic activity at rest appeared as sporadically occurring bursts. When aroused by the experimenter or asked to perform mental arithmetic, there was a pronounced increase in the sympathetic outflow. A strong positive relationship between the ongoing activity in Pacinian corpuscles and the amount of sympathetic outflow was observed in these normal subjects. In some cases the increased firing of the Pacinian unit outlasted the duration of the recorded sympathetic bursting activity.

The fact that activity in afferent and sympathetic nerve fibers supplying the same target area can be studied synchronously may help to clarify the role of the sympathetic nervous system in sensation. In addition, investigations of specific pathophysiological problems in clinical pain may be facilitated.

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245.15 RESPONSES OF JOINT CAPSULE AFFERENTS RELATED TO THE COMPONENTS OF STRAIN IN ISOLATED KNEE CAPSULE. Peter Grigg. Department of Physiology, University of Massachusetts Medical School, Worcester, MA 01605.

Afferents are recorded from the posterior articular nerve, which innervates the posterior region of the capsule of the cat knee joint. The capsule is excised from the knee and studied as a 2-dimensional sheet in an apparatus in which it is subjected to controlled deformations. In previous studies utilizing this preparation (Grigg, P. and Hoffman, A.H., J. Neurophysiol., 47, 41-54, 1982), discharges of afferents could be related only to estimates of stresses at the locus of the receptor in the capsule. In the present study, neuronal discharges are recorded while mechanical measurements are made of actual capsule deformations (strains) at the receptor location in the capsule. Strain measurements are made by placing an array of markers around the receptor locus and measuring the positions of those markers before and during a deformation of the capsule. Marker positions are measured by video frame grabbing a television image of the decorated capsule surface. Strains are computed from measurements of marker displacements, using finite element theory; solutions are obtained for shear strain and for tensile strains along axes corresponding to the bone long axis and the orthogonal axis of the knee. In preliminary results, activity of afferents was related to all components of strain, with the best correlations between neuronal discharge and shear strain. Supported by NIH grant NS-10783.

245.16 LOCALIZATION AND MORPHOMETRIC ANALYSIS OF NEURONS INNERVATING MASTICATORY MUSCLE AFFERENTS IN CATS. N. F. Capra, K. V. Anderson, Y. Khawaji*, and M. Morrison*. Section of Craniofacial Biology, Univ. Med. Center, Jackson, MS 39216.

The nucleus of the mesencephalic root of the trigeminal nerve (mesencephalic nucleus) is composed of neurons that innervate sensory receptors in the muscles of mastication, the periodontal ligaments, and the oral mucosa. As part of a continuing effort to describe the specific anatomical localization and morphological features of neurons in the mesencephalic nucleus that innervate specific craniofacial structures, unilateral focal injections of Horseradish peroxidase (HRP, Sigma VI, 40% solution) were made into the masseter muscle and into the contralateral temporalis muscle in five cats. The animals were allowed to survive 48-72 hours. Serial frozen sections (40 m. thick) were made through the brainstem from the caudal boundary of the trigeminal motor nucleus to the posterior commissure. The sections were processed for HRP reactivity according to the methods described by Mesulam (J. Histochem. Cytochem. 26:106, 1978). Labeled neurons in the mesencephalic nucleus of each side of the brainstem were examined with a computer-based image analysis system and their locations were mapped on transverse and sagittal drawings of the cat brainstem. The number of labeled perikarya resulting from the injection of either muscle was quite variable, but more cells were usually observed ipsilateral to the side of the injected masseter muscle. The diameter of the labeled cells in both muscles ranged from 13 m. to 60 m. Cells innervating both the masseter and temporalis muscles were scattered throughout the rostrocaudal extent of the mesencephalic nucleus. However, there was a marked tendency for the masseter muscle to receive fibers from perikarya located in the rostral midbrain. These cells were located along the dorsal and lateral borders of the periaqueductal gray ventral to the superior colliculus. Quantitative data obtained from the labeled neurons will be compared with control data to further characterize cells that convey primary afferent information from the jaw-closing muscles.

245.17 SUPERPOSITION OF IMPULSE SEQUENCES IN AN AFFERENT UNIT MODEL
M. D. Goldfinger. Dept. of Biology & School of Medicine University of Missouri, Kansas City, MO 64110

In many mechanoreceptor afferent units, the parent axon innervates several end-organs. The present work studies the contribution of each terminal to the discharge pattern of the parent axon; details of the model are given elsewhere (1,2).

I. Strictly periodic inputs: Each terminal is driven independently at a different period. As the number of driven terminals (N) is increased, both the average stimulus rate to the entire afferent unit (Fs) as well as the average parent axonal firing rate (Fa) increase. For a given N: Fa<Fs, due to the axonal absolute refractory period (ARP); Fs-Fa is a function of N. With highest intensity stimuli over a range of N(4-25) : $Fa(N)\alpha$ Log N; the slope (60-100) varies inversely with the difference between the individual terminals' input periods. The impulse sequence of parent axonal discharge is a function of stimulus intensity, which is expressed in terms of the shortest period P at which 1:1 firing from 1 terminal is supported. For larger N, maximal stimulus intensity (P=ARP) yields a basically constant Expectation Density (ED) subsequent to ARP(1). Lower intensities (P>ARP) yield EDs consisting of: deadtime (=P), damped oscillatory transients at multiples of P, and a steady-state plateau.

II. Poissonian stimulation: The waiting time for the next successive stimulation of a given terminal is determined by equiprobable selection of intervent intervals generated by a stable Poisson process (nuclear disintegration; mean interval = 33 ms). For highest intensity stimuli (P=ARP), Fa increases non-arithmetically with N. The ED consists of: a deadtime (=ARP), a single peak without undershoot (large N), and plateau. With progressively lower stimulus intensities (P>ARP), the ED consists of: a deadtime (=P), a series of increasingly damped oscillatory transients occuring at multiples of P, and a steady-state plateau. Such ED transients are attributable to the threshold elevation during the 1st P msec of antidromic recovery cycles.

Similar ED oscillatory transients were described for the G-hair afferent unit discharge during continuous airjet stimulation (3), but were elicited only by increasingly higher intensity stimuli. This difference implies that the mechanism of impulse initiation by the G-hair afferent unit during continuous aperiodic stimulation includes neither antidromic impulse propagation nor instantaneous resetting of terminal threshold.

REF's: (1) Goldfinger, Soc. Neurosci. Abst. 7:948, 1981.
(2) Goldfinger & Fukami. J. Neurophysiol. 45:1096-1108, 1981.
(3) Goldfinger & Amassian. J. Neurophysiol. 44:961-977, 1980.

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245.18 RELATIONSHIP OF AFFERENT NERVE ACTIVITY IN THE PELVIC PLEXUS WITH PRESSURE, LENGTH AND WALL STRAIN IN THE URINARY BLADDER OF THE CAT.
J.W. Downie and J.A. Armour. Departments of Pharmacology and Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

Traditional descriptions of vesical afferent activity relate firing to both distension and contraction. However this relationship is not always strong presumably due to local variations in bladder wall response. We sought to determine whether the size of receptor fields of vesical afferents would provide a stronger correlation with afferent traffic. In 10 chloralose-anesthetized cats with acute bilateral L_7-S_3 rhizotomies, fine cannulae were positioned at the iliac trifurcation at laparotomy for intra-arterial (i.a.) injection of drugs. The bladders were cannulated for filling and pressure monitoring via urethrostomies. In 5 cats a wide-bore tube was inserted into the bladder apex and connected to a reservoir for maintaining constant bladder pressure. Nerve fibers in the pelvic plexus were cut centrally and dissected into fine filaments in which only a few units were active. The receptor fields of the afferents were determined by probing the surface of the bladder and in some experiments by electrical stimulation. Length-measuring ultrasonic probes were sewn onto the bladder surface around the receptor field and a strain guage arch was secured to one side of the field. The conduction velocities of the afferents were 2 - 8 m/s. The 12 units analyzed under isometric conditions had widely different thresholds. They fired during a ramp increase in volume but their rates of firing fell during the maintained volume and became episodic. Episodes of firing were not always correlated with variations in pressure, wall strain or length. On withdrawal, rates of firing during the plateau phase were higher than for comparable pressures during filling. Four of 5 units tested responded during a brief contraction elicited by i.a. 4,4-dimethylphenyl piperazinium (DMPP). The 12 units analyzed under isotonic conditions showed a burst pattern of firing around threshold pressures but the pattern became more sustained as pressure was increased. Three units became silent at higher pressures. Firing rates were not equivalent at comparable pressures on withdrawal. Only 2 of 10 units tested responded during DMPP-induced contraction under isotonic conditions although there was a dramatic shortening in the receptive field. Under isometric conditions where there was minimal shortening, 8 of the 10 units responded. Afferent activity does not correlate directly with pressure, wall strain or receptor field dimension. Thus bladder mechanoreceptors behave in a more complex manner than hithertofore has been considered.
(Supported by MRC Canada).

245.19 STIMULATION OF RABBIT CORNEAL NERVES BY ACETYLCHOLINE AND NICOTINE.
D.L. Tanelian, R.W. Beuerman and M. Young*. Div. of Ophthalmology, Stanford Univ. Med. Ctr., Stanford, CA 94305 and LSU Eye Center, LSU School of Medicine, New Orleans, LA 70112.

The rabbit corneal epithelium contains high concentrations of acetylcholine (ACH) and the enzymes for its synthesis and degradation. However, no functional or physiologic role has been attributed to this cholinergic system. These experiments have investigated the cholinergic pharmacology of the corneal sensory nerves. Albino rabbits were anesthetized with urethane and the long ciliary nerve was obtained for action potential recording. Acetylcholine (.01-5.0 mg/ml) nicotine (10^{-5} to 10^{-3} M), bethanechol (5 mg/ml), carbachol (10 mg/ml), d-tubocurarine (3 mg/ml), atropine (10^{-6} to 10^{-3} M), and physostigmine (7.5 mg/ml) were made up in isotonic saline just before use. After obtaining an identifiable single action potential recording, a test drug was instilled at 33°C into a one ml chamber covering the cornea. Twenty-four units were tested for the effects of mechanical and drug stimulation.

Acetylcholine increased the action potential frequency in a graded fashion from x=5 Hz, n=8 at .01 mg/ml to x=48 Hz, n=8 at 1 mg/ml, followed by several minutes of desensitization. Bethanechol, a muscurinic agonist, was not stimulatory, whereas carbachol produced mild excitation. Nicotine, 10^{-5} to 10^{-4} M, also produced a gradual increase in action potential discharge similar to that of ACH. However, at 10^{-3} M, excitation was followed by a period of blockade. Physostigmine was excitatory for several seconds before producing a blockade. Both tubocurarine and atropine abolished the excitatory response to all concentrations of ACH and nicotine for more than one hour. Mechanical stimulation and other pharmacologic agents [histamine (2-20 mg/ml), bradykinin (10^{-5} M), substance P (10^{-4} M), and capsaicine (1%)] did not excite these units.

In contrast to these cholinergic units, the corneal mechanoreceptors (Tanelian and Beuerman, Soc. Neurosci. Abs. Vol. 7, p. 271, 1981) were not stimulated by ACH or nicotine, and their mechanical sensitivity was not impaired by atropine or tubocurarine. The selective nature of cholinergic stimulation may have functional significance in the rabbit cornea.

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245.20 NEURONS IN THE AREA POSTREMA PROJECT TO THE DORSO-LATERAL PARABRACHIAL NUCLEI IN RAT.
D. van der Kooy and L.Y. Koda (SPON: P.E. Garfinkel). Neurobiol. Res. Group, Dept. of Anatomy, Univ. of Toronto, Toronto, Canada M5S 1A8 and Behav. Neurobiol. Lab, Salk Institute, San Diego, CA 92138.

The area postrema is a circumventricular organ (on the blood side of the blood-brain barrier) situated at the caudal end of the IV ventricle. Historically the area postrema has been seen as a neuron-poor area. This raised the question of how information (blood-borne signals) are transferred from the area postrema to other parts of the brain. We reanalyzed this problem by making small injections of the anterograde tracer WGA-HRP into the area postrema. In agreement with other studies some projections were seen to the solitary nucleus (immediately ventral to the area postrema) as well as sparse projections to the pontine central gray. Most striking, however, was the substantial anterograde label in a restricted portion of the parabrachial nuclei (the region immediately dorsal to lateral portion of the brachium conjunctivum in the rostral pons). Because the solitary nucleus adjoining the area postrema is known to project to the parabrachial nucleus, we attempted to confirm the area postrema-parabrachial pathway using retrograde tracing. Injections of True Blue into the dorso-lateral parabrachial nucleus produced large numbers of retrogradely labeled cell bodies throughout the area postrema. The labeled cells were only slightly more numerous on the side of the area postrema ipsilateral to the True Blue injection. Retrogradely labeled cell bodies were also seen with an ipsilateral predominance in certain sub-nuclei of the solitary complex, including the internal solitary zone and dorsal and medial divisions of the medial solitary nucleus.

Given the recently demonstrated projections from the vagus nerve and hypothalamus to the area postrema, the neurons in the area postrema projecting to the parabrachial nuclei might be viewed simply as displaced solitary nucleus cells. However, the large number of cells situated bilaterally throughout the area postrema that project to a restricted region of the parabrachial nuclei would seem to argue against this. Regardless, the area postrema - parabrachial pathway demonstrated here provides an important route whereby blood-borne information (that cannot cross the blood-brain barrier) can be communicated to a multitude of brain regions.

246.1 NUMBER AND SIZE DISTRIBUTION OF L7 DORSAL ROOT AXONS AND GANGLION CELLS AFTER TRANSECTION OF THE SCIATIC NERVE IN ADULT CATS. Håkan Aldskogius* and Mårten Risling* (SPON: G. Grant). Dept. of Anatomy, Karolinska Institutet, Stockholm, Sweden.

Following sciatic nerve transection in kittens, a marked reduction in the number of L7 dorsal root (DR) axons and ganglion (DRG) cells takes place (Risling et al., Exp. Neurol. 67: 265, 1980, Aldskogius and Risling, Exp. Neurol. 74: 597, 1981). In the present study the effect of sciatic nerve transection on the L7 DR and DRG in the adult cat have been investigated. The left sciatic nerve was transected and a few cm of its distal stump resected. 35, 90 and 190 days p.o. the animals were perfused with phosphate-buffered, 5% glutaraldehyde. L7 DR and DRG were removed bilaterally. Roots were embedded in Vestopal and prepared for light and electron microscopy. Cross-sectional root area was determined planimetrically. Mean density of myelinated axons was estimated. The total number of myelinated axons could then be calculated. The ratio unmyelinated/myelinated axons was determined for the central portion of the root by direct counting in the electron microscope. External diameters of myelinated fibers were measured on photomicrographs with a particle size analysator. DRG were embedded in paraffin, sectioned serially at 10 µm, and stained with cresyl violet. Neuronal nucleoli were counted in every 10th section. A correction factor for split nucleoli was used, and the number of DRG cells calculated. Perikaryal sizes were determined by measuring major and minor axes of DRG cells with a visible nucleolus on photomontages of randomly chosen sections. A series of normal animals was prepared and examined in the same way as described above.

In normal animals, the number of DR axons and DRG cells was very similar bilaterally in each individual. However, the number of DRG axons - at the root level halfway between the DRG and spinal cord - exceeded the number of DRG cells significantly, probably due to branching of DR axons close to the ganglion. In the experimental animals, a slight reduction of the number of myelinated and unmyelinated axons was found ipsilateral to operation. In the corresponding DRG, the number of neurons was reduced by about 30%. The size distribution of myelinated fibers and DRG neuronal perikarya was shiftet towards smaller sizes on the operated side. This change seemed to be the result of atrophy of larger fibers and neurons, respectively, rather than a selective loss of such fibers or cells.

The findings indicate that sciatic nerve transection in adult cats results in a marked loss of DRG cells with no preference for a certain size class. However, the number of axons in the corresponding dorsal root appears to be much less affected. Possible resasons for this discrepancy are currently under investigation. (Supported by the Swedish Med. Res. Council, projs. 05420 and 03761).

246.2 ACCELERATED REGENERATION OF CRUSHED HYPOGLOSSAL NERVE BY TESTOSTERONE. W.H.A. Yu and M.C. Yu*. Departments of Anatomy, Mount Sinai School of Medicine, New York, NY 10029, and New Jersey Medical School, Newark, NJ 07103.

We have shown previously that administration of testosterone to rats following the transection of the hypoglossal nerve promotes axonal growth. However, irrespective of the treatment the projections of regenerating fibers into the muscles were non-specific (Exp. Neurol. 71:431; 77:in press). In the present study we have examined the effect of testosterone on axonal growth after crushing the nerve and the specificity of re-innervation.

Sprague-Dawley rats (160-180 g) of both sexes were used. Under anesthesia the right hypoglossal nerve was crushed proximal to its bifurcation. The rats were then divided into two groups and treated as follows: Group #1: 5 mg of testosterone propionate (TP), twice a week, i.m.; Group #2: a similar regimen of injections with the oil vehicle alone. Groups of rats were killed at the 7th, 8th, 10th, and 12th postoperative (PO) days, respectively. Twenty-four hours prior to killing 50 ul of a 10 % horseradish peroxidase (HRP) solution was injected into the tongue. The rats killed at the 12th PO day had the medial branch of the right hypoglossal nerve severed prior to the HRP injection. The brain stems containing the hypoglossal nuclei were sectioned transversely at 50 um. Alternate serial sections were stained with the tetramethylbenzidine-H_2O_2 method. The presence of HRP labeled neurons in the right hypoglossal nucleus was taken to indicate the arrival of regenerating fibers to the tongue. The total number of labeled neurons in the right nucleus was expressed as the percentage of that of the left nucleus and referred to as the "percentage regeneration".

HRP labeled neurons were first noted in the right nucleus in some rats 7 days after the lesion. At the 8th PO day about 40 % regeneration was seen in all the rats. By the 10th PO day the TP and control groups had 100 % and 80 % regeneration, respectively. The difference between the two groups was statistically significant ($p<0.01$). Following the transport of HRP by the lateral branch of the regenerated nerve, the labeled neurons were located exclusively in the dorsal subnuclear group similar to that found in the intact animals. These data suggest that testosterone accelerates axonal growth following crush injury. The tomatotopic organization of the nucleus of the crushed nerve was unaltered, and was attributed to the intact peri- and endoneurial sheaths which prevented disorientation of regenerating fibers.

246.3 CONTRACTILE PROPERTIES OF SKELETAL MUSCLE UNDERGOING REINNERVATION: EFFECTS OF TESTOSTERONE AND CASTRATION. S.P. Yeagle, R.F.Mayer, S.R.Max. Dept. of Neurology, Univ. of Maryland, School of Medicine, Baltimore, Maryland 21201.

We studied the contractile properties of skeletal muscle to establish the temporal sequence of events during reinnervation following nerve crush. Male rats were examined 8-21 days after crushing the peroneal nerve approximately 1.0 cm from its entrance into the extensor digitorum longus (EDL) muscle. Isometric contractile properties of EDL measured in vivo after nerve stimulation included twitch tension, twitch contraction time to peak, tetanic tension, twitch-tetanus ratio, posttetanic potentiation of twitch tension (PTP), and optimal frequency of stimulation. The earliest signs of functional reinnervation were observed 8-9 days after nerve crush, with twitch tensions 1-2% of control levels (control: 0.18 g/g body wt.), prolonged twitch contraction times (25-28 msec vs 14-15 msec for control), and tetanic tensions that declined rapidly during 20 Hz stimulation. By day 10-11 twitch tensions were 10-20% of control, twitch contraction time declined to 21-26 msec, and tetanic tensions were 4-9% of control (control: 0.86 g/g body wt.) with optimal stimulation frequency of 58-64 Hz (control: 198 Hz). The twitch-tetanus ratio at this time (0.57-0.61) was 3 times that of controls (0.19) and a 5 second, 20 Hz stimulus train produced no PTP of muscle twitch (vs. 52% for control). Over the next 9 days there was gradual return of all contractile properties toward control values; the relative rate of return was twitch tension>twitch contraction time >twitch-tetanus ratio>tetanic tension>optimal frequency of stimulation>PTP. For any given animal, the twitch-tetanus ratio during this period was highly correlated with the degree of functional recovery of neurally-evoked muscle tension and was found to be the best index of the state of muscle reinnervation.

Testosterone reportedly enchances the rate of regeneration of the hypoglossal nerve following transection (Exp.Neurol. 71:431, 1981). Accordingly we assessed the effects of testosterone and castration on nerve regeneration and muscle reinnervation. Neither treatment significantly altered the rate or extent of recovery of any of the measured contractile properties during the 14 day period which was studied ($P>0.05$). The absence of differences among normal, testosterone-treated, and castrated rats leads us to conclude that testosterone does not significantly influence the rate or extent of reinnervation of the EDL following nerve crush. The study of muscle contractile properties and their temporal sequence of recovery will be especially valuable for future evaluation of agents that may influence reinnervation of denervated muscle. Supported by grants from NIH (NS 15760) and NASA (NAG2-100).

246.4 THE EFFECT OF AXONOTMESIS AS A THERAPY FOR NEUROTMESIS. H.R. Koerber and K.W. Horch. Dept. of Physiol., Univ. of Utah Col. of Med., Salt Lake City, UT 84108.

The effect of proximal nerve crush on promoting regeneration after unrepaired nerve transection lesions was studied in feline cutaneous nerves. In 25 cats the sural nerve was transected midway between the ankle and the knee. Following interlesion intervals of 0 days, 2 wks., 1 mo., 2 mo., 4 mo., and 6 mo., the nerves were crushed 1 cm proximal to the original lesion. The animals were then allowed to survive for 9 mo. to allow sufficient time for all regenerating fibers to reach the skin.

In order to compare the quality of regeneration following nerve transection with that after nerve transection and proximal nerve crush, four measures of regeneration success were made: (1) the number of fibers crossing the neuroma and regenerating down the distal stump; (2) the conduction velocity of fibers in the distal stump relative to their conduction velocity proximally; (3) the number of these fibers reinnervating cutaneous mechanoreceptors; and (4) the number of cutaneous type I mechanoreceptors present after regeneration.

Preliminary results of data collected to date indicate that: (a) crushing at the time of the transection produced no significant improvement in any of the measures and caused a significant impairment in measurement 2, and (b) proximal nerve crush 6 mo. after transection produced significant improvement in measures 1 and 2 and a measurable but non-significant improvement in 3 and 4. Data for the remaining interlesion intervals is currently being collected.

246.5 LOW INTENSITY DIRECT ELECTRICAL CURRENTS FACILITATE THE RATE OF SCIATIC NERVE REGENERATION IN THE ADULT RAT. M. A. Mullen* and B. H. Pomeranz. Dept. of Zoology, Univ. of Toronto, Canada, M5S 1A1.

Preliminary studies revealed that, after sciatic nerve injury, regeneration of motor axons into the ventral interossei muscles of the adult rat hindpaw followed a reliable timecourse. Following a standarized sciatic nerve crush, high in the thigh region, this reinnervation was complete within 10 - 12 days post-lesion, in control animals. In no control did reinnervation occur before 8 days; therefore, 7 days post-lesion was chosen as an appropriate window for testing in order to determine whether or not minute direct electrical stimulation (DC 1μA, 30 minutes daily for 6 days) would enhance the rate of regeneration.

For the present study, reinnervation was determined in an acute experiment at 7 days, utilizing an electromyography (EMG) paradigm. Subjects received sciatic lesions and were then randomly assigned to one of three groups: (1) DC^- 1μA stimulation (cathodal stimulation of the hindpaw), (2) DC^+ 1μA stimulation (anodal stimulation of the hindpaw), and (3), Controls (who received no treatment).

The acute phase of the experiment entailed assessment of reinnervation with extra-cellular, multiple unit recordings of 6 muscle groups (tibialis anterior, medial and lateral gastrocs, abductor digiti quinti and 2 groups of ventral interossei). Stainless steel recording electrodes detected EMG's elicited by direct stimulation of the sciatic, above the crush site, using Ag/AgCl stimulating electrodes. The threshold, latency, frequency following ability and fatigue time for each muscle group was determined.

Results indicate that 9/10 DC^- 1μA animals demonstrated reinnervation of the ventral interossei as compared with 0/10 Controls and 1/10 DC^+ 1μA animals ($p < 0.01$). In addition, DC^- 1μA subjects displayed a trend toward lower thresholds, shorter latencies and prolonged fatigue times in the calf muscles tested when compared with DC^+ 1μA and Control counterparts. Such results suggest that small, negative (cathodal) direct currents may play a positive role in the rate and quality of regeneration following sciatic nerve injury.

246.6 REDUCED CHEMOSENSORY DISCHARGE FOLLOWING REINNERVATION OF THE CAROTID BODY BY FOREIGN AXONS. L. Stensaas, B. Dinger* and S. Fidone. U. of Utah, Sch. of Med., Salt Lake City, UT 84108.

A fundamental issue in sensory physiology is whether foreign regenerating axons will associate with preneural cells and consequently adopt a new functional modality. An analysis of the morphological and physiological correlates of reinnervated end-organs may provide insight into the normal transducer function of such preneural cells. Two types of preneural cells form lobules in the arterial chemosensory tissue of the carotid body. Afferent fibers from the carotid sinus nerve (CSN), a branch of the IX^{th} cranial nerve, penetrate the lobules to form either unmyelinated axons enveloped by type II (sustentacular) cells, or specialized terminals in synaptic apposition with type I (glomus) cells. An unsettled question concerns the contribution to chemosensory transduction made by afferent axons and terminals, relative to that of the type I and type II cells.

Utilizing surgical cross-anastomosis we have reinnervated the cat carotid body with foreign axons from the lingual branch (LN) of the IX^{th} cranial nerve which normally innervate tongue mechanoreceptors and taste buds. We previously reported (Neurosci. Absrcts. 6:94, 1980) a severe reduction in the incidence of specialized nerve terminals apposed to type I cells following cross-reinnervation, but normal numbers of nerve terminals reformed from regenerating CSN axons. Current studies evaluate both the physiological and ultrastructural alterations that occur consequent to reinnervation of the chemosensory tissue. The size of the summated discharge evoked following reinnervation by the transected CSN was indistinguishable from normal, while recordings from foreign reinnervated carotid bodies revealed large reductions in the chemosensory response elicited by hypoxia, NaCN and acetylcholine. The magnitude of the decrease closely paralleled the reduction in the incidence of nerve terminals. In contrast, the number of intralobular unmyelinated axons (unapposed to type I cells) tended to be elevated in reinnervated carotid bodies. Thus, penetration of the lobules by foreign axons, without the formation of appositional relationships on type I cells, does not produce the conditions necessary for normal chemotransduction.

The available data suggest that particular axons possess specific properties which allow them to form appositional relationships with type I cells, and furthermore they support the hypothesis that such appositions, and therefore the type I cells, are important for the development of arterial chemosensation. Supported by USPHS Grants NS 12636 and NS 07938.

246.7 PHYSIOLOGICAL AND MORPHOLOGICAL OBSERVATIONS OF CROSSED VENTRAL NERVE ROOTS IN THE SACRAL SPINAL CORD OF THE CAT. Neal Shonnard* and Caroline Wakefield, Dept. of Anat., Univ. of Nevada, Sch. of Med., Reno NV 89557.

Previous studies of anastomosis of lumbar or sacral nerve roots, either by straight reunion or by transposition of roots from different spinal levels, have shown functional regeneration to lower limb musculature. These studies have prompted further experimental work on anastomosed nerve roots as a possible means of gaining functional recovery following spinal cord or nerve root injury. In addition, the use of fine monofilament nylon suture (11-0 Ethicon) for suture of ventral root sheaths facilitates the operative procedure and provides incentive for clinical application. The first sacral (S_1) nerve root innervation of the gastrocnemius muscle was selected for study because of easy access to this long nerve root. We reconstructed the ventral roots of S_1 so that the right root innervated the left gastrocnemius muscle (cross innervation). Regeneration of alpha and gamma motor neurons was studied by physiological and horseradish peroxidase (HRP) methods. The animals were sacrificed at 5 and 8 months. Right S_1 nerve roots were stimulated with rectangular electrical pulses of 0.5ms duration at varying intensities and the electrical activity of left gastrocnemius muscle was recorded with 90μ diameter insulated stainless steel wires inserted into the muscle through a 21 gauge hypodermic needle. Electrical stimulation of right S_1 roots evoked action potentials in the left gastrocnemius muscles at 3, 5 and 8 months. Localization of stimulation to the affected root was confirmed by neuronal blockade with 2% Lidocaine in 0.9%NaCl. After electrophysiological confirmation of regeneration of the nerve root, HRP was injected into the left gastrocnemius muscle. Alpha and gamma motor neurons in the right S_1 motor cell column were labeled by HRP. Preliminary analysis of the tissue showed motor neuron labeling in the appropriate cell column. Approximately 88% of all labeled motor neurons were alpha motor neurons and 12% were gamma motor neurons. These results show that functional regeneration occured after crossing nerve roots. Supported by the Research Advisory Board of the University of Nevada at Reno.

246.8 GUSTATORY TROPHIC ACTION OF ARTERIAL CHEMOSENSORY NEURONS. B. Dinger*, L. Stensaas and S. Fidone. U. of Utah, Sch. of Med., Salt Lake City, UT 84108.

Taste buds consist of groups of specialized cells embedded in squamous epithelium. Interruption of the afferent axons supplying cat taste buds results in their disappearance within two weeks, while regeneration of the gustatory fibers induces the reformation of the taste apparatus via cellular proliferation and differentiation. Over the past 25 years numerous cross-reinnervation experiments have shown that taste buds do not reappear under the influence of motor or cutaneous sensory axons. However, our recent finding (Neurosci. Lett. 27: 285-289, 1981) that functional taste buds reappear in cats following cross-anastomosis of the carotid sinus nerve (CSN) to the lingual nerve (LN) suggests that a restricted group of foreign axons are able to trophically influence the differentiation of gustatory epithelial cells.

Additional studies have attempted to determine the normal functional modality of the foreign CSN fibers which reinnervate taste buds. The CSN contains arterial chemosensory and barosensory axons from the carotid body and carotid sinus, respectively. Stimulation of the carotid body reflexly increases pulmonary ventilation and blood pressure while activation of the barosensory axons evokes respiratory inhibition and reductions in blood pressure. Therefore, we studied the cardiopulmonary reflex changes initiated by mechanical and gustatory stimulation of circumvallate papillae several months following surgical cross-union of the carotid and lingual branches of the IX^{th} cranial nerve. Mechanical stimulation consistently elicited decreases in blood pressure and also occasionally inhibited respiration; excitation of respiration was never evoked by mechanical stimuli. These data suggest that regenerating barosensory, and not arterial chemosensory, fibers reassociate with tongue mechanoreceptors. In contrast, gustatory stimulation consistently evoked respiratory excitation. Increases in both tidal volume and respiratory frequency were observed in response to 1M NH_4Cl, 0.02 quinine-HCl and 2 or 4M NaCl; sucrose, a poor stimulator of cat taste buds, did not appreciably alter breathing. Respiration and blood pressure of normal animals were unaffected by mechanical or gustatory stimuli.

The results indicate that taste buds are capable of increasing respiratory drive following nerve cross-union with the CSN, and they imply that regenerating foreign arterial chemosensory and barosensory axons become segregated between taste bud and mechanoreceptor preneural sensory structures, respectively. Furthermore, we conclude that arterial chemosensory and gustatory chemosensory axons are similarly endowed with a trophic component essential for the induction and maintenance of taste buds.

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246.9 FURTHER CHARACTERIZATION OF NEURONOTROPHIC FACTORS ACCUMULATING IN VIVO WITHIN NERVE STUMP-CONTAINING SILICONE CHAMBERS. F.M. Longo*, S.D. Skaper, M. Manthorpe, G. Lundborg* and S. Varon (SPON: G. Barbin). Dept. Biol., Sch. of Med., Univ. Calif., San Diego, La Jolla, CA 92093.

Neuronotrophic Factors (NTFs) accumulate in vivo within a regeneration system developed by Lundborg and coworkers. Rat sciatic nerves are transected and their proximal and distal stumps sutured into the openings of cylindrical silicone chambers. Anatomic regeneration has been demonstrated across 10 mm long chambers containing both stumps, although little or no neuritic outgrowth occurs in chambers omitting the distal stump or exceeding the 10 mm length (Lundborg et al., Exp. Neurol. 76: 361-376, 1982). Chambers containing both proximal and distal stumps, or either stump with the opposite chamber-end ligated, accumulate a clear fluid within hours of implantation. This fluid contains considerable neuronotrophic activity (assayed in embryonic neuronal cultures) for types of neurons which contribute neurites to the sciatic nerve (sensory, motor and sympathetic). In chamber fluid collected 1 week after chamber implantation, NTF levels directed to different neuronal types varried independently from one another with different nerve insertions, suggesting that these activities reside in separate factors. The effects of heat, dialysis or trypsin treatments on fluid NTF activities directed to all 3 types of neurons suggest that these activities are associated with protein. Generally higher titers of all NTFs were found in chambers containing either or both nerve stumps than in nerve-free chambers. NTF levels directed to sensory neurons peaked by several hours post-transection, while NTF titers directed to motor and sympathetic neurons peaked after several days. These differential temporal dynamics indicate further that these respective trophic activities reside in different factors. Fluid collected from chamber arrangements allowing minimal neuritic growth did not always contain correspondingly lower titers of NTFs. The relevance of these NTFs to in vivo neuronal regeneration remains to be determined.

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246.10 IN VIVO MODEL FOR NERVE REGENERATION: TEMPORAL PATTERN OF AXONAL REGROWTH. Lawrence R. Williams, Frank M. Longo*, Göran Lundborg* and Silvio Varon. Dept of Biol. Sch. of Med., Univ. of Calif. San Diego, La Jolla, CA 92093.

Lundborg and coworkers have developed an in vivo model where the proximal (P) and distal (D) stumps of a resected rat sciatic nerve are inserted into opposite ends of a silicone tube, allowing a 10 mm gap between them, i.e. a PD 10 chamber (Lundborg et al., 1982, Brain Res. 232: 157; Exp. Neurol. 76: 361). The model lends itself to manipulations of the intrachamber microenvironment which might affect axonal regeneration. Screening of the impact on nerve regeneration by any such manipulation requires detailed knowledge of the temporal advance of regenerating axons in the chamber and selection of simplified procedures to afford appropriate monitoring criteria. As a first step, we have examined, by light microscopy, the progress of axon regeneration at 2 mm intervals (transverse sections, S_1-S_9), 1-4 wks post-implantation (PI). At 1 wk PI, a translucent structure extended from both stumps across the gap. It may be composed of fibrin and collagen with randomly distributed hematogenous cells. By 2 wks PI, Schwann cells, fibroblasts and capillaries had invaded the chamber from both stumps. Regenerating axons were seen at S_3 and myelination of axons was apparent at S_1. By 3 wks PI, axons had regenerated to S_7; regeneration units or compartments containing large axons were observed. Axons had regenerated across the entire chamber by 4 wks PI. A proximal-distal gradient of myelin formation was apparent as was the case for nerve maturation. Electron microscopy is being used to ascertain the extent of axonal regeneration beyond these light microscopic results. The anterograde axonal transport of radiolabeled proteins is being explored as an alternate, more convenient monitoring technique. Selected transverse sections (S_3 and S_7 at 3 wks PI) were used to investigate whether the requirement of a distal nerve for regeneration within the chamber can be met by a 2 mm piece of nerve separated from its end organs. The nerve piece was found to be sufficient, permiting regeneration across the chamber.

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246.11 REGENERATION OF THE PERIARTERIAL ADRENERGIC PLEXUS AFTER ANASTOMOSIS OF THE CAROTID ARTERY OF THE RABBIT. S. Finn*, M.S. Beattie, J.C. Bresnahan, L.A. Gray* and W.E. Hunt. Div. of Neurosurg. and Dept. of Anat., The Ohio State Univ., Columbus, OH 43210.

Section and anastomosis of arterial vessels is a frequent surgical procedure which necessarily interrupts the periarterial adrenergic plexus. The present experiment was designed to evaluate the temporal course of the degeneration and potential regeneration of this nerve fiber plexus following such a procedure.

18 white rabbits (400-600gm) were used. After exposure of the left common carotid, vascular clips were applied 12mm apart and the artery was transected. The cut ends were anastomosed with 10-0 nylon suture. In control animals only the clips were applied. The vessels were harvested at 1, 12 and 24 hrs., 1, 3 and 6 wks. post-anastomosis. After anesthesia and intracardiac perfusion with oxygenated ringer's lactate, the arteries were cut longitudinally in situ. The tunica intima and media were gently dissected from the rest of the vessel wall. The tissue was treated for the demonstration of catecholamines by the Falck-Hillarp, or Eranko and Raisanen's (J. Histochem., 14: 690, 1966) method. The vessel was glued to a glass slide so the opened vessel remained flat while drying, was covered with Entellan, coverslipped, and observed under the fluorescence microscope.

Immediately after anastomosis, the fluorescence between the clips was patchy and reduced in brightness. In the regions distal to the clips, a very few thick fibers were quite bright and the varicosities close to the clips appeared brighter than normal and slightly enlarged. By 12-24 hrs. the area between the clips was nearly devoid of fluorescence, while distal to the clips, several very large, bright fibers were observed, some displaying retraction bulbs. Fibers of normal size were not present in this area but there was a transition to an area of normal appearing fibers. After 1 to 3 wks. in the area between the clips, 5 out of 7 animals displayed an occasional fiber with a few very fine, long thin processes emanating from the tip. 6 wks. post-anastomosis, a rather rich plexus (although not as dense as normal) of adrenergic fibers was observed between the clips and approached the anastomosis site in one animal. In a second animal, this plexus was present on one side, but this animal had a mural clot on the other side (possibly inhibiting the regrowth on that side).

These results substantiate a recent report by Wada (J. Microsurg., 3: 20-27, 1981) of regeneration of the plexus in the femoral artery of rabbits after arterial grafts. The use of whole mounts of rabbit carotid artery in the present experiment allows for further description of the course and nature of this regeneration. (Supported by NS-10165, NS-14457; MRDF Funds, Department of Surgery; and Roessler Foundation.)

246.12 LACK OF MUSCLE REINNERVATION AFTER NERVE CRUSH IN HIBERNATING GROUND SQUIRRELS. M. Kalia, S.S. Deshpande*, T. Ducker* and E.X. Albuquerque. Department of Physiology, Hahnemann Medical College, Philadelphia, PA and Depts. Pharmacology and Experimental Therpeutics and Neurosurgery, Univ. of MD School of Medicine, Baltimore, MD

During hibernation (5-7° C) the excitability of skeletal muscle in the 13-lined ground squirrel (C. tridecemlineatus) is maintained and after denervation the distal nerve stump does not degenerate for at least 30 days (Albuquerque et al., Exp. Neurol., 62, 1978) provided the animal remains in deep hibernation. During hibernation axonal transport is significantly reduced (Boegman and Albuquerque, Exp. Neurol., 68, 1980) or blocked (Bisby and Jones, Exp. Neurol., 61, 1978). To study the extent of nerve regeneration during hibernation, the peroneal branch of the sciatic nerve was crushed with a fine forceps 8-10 mm (short nerve segment, SNS) or at 30-33 mm (long nerve segment, LNS) from its entrance into the extensor digitorum longus (EDL) muscle. A similar operation was performed in nonhibernating animals. Regeneration at the crush site was followed morphologically by retrograde transport of horseradish peroxidase (HRP) through the crush region and the reinnervation of the EDL muscle was studied using conventional electrophysiologic techniques. Thirty days after nerve crush in nonhibernating animals, the surface fibers of the EDL muscle were depolarized by 8-10 mV with LNS and by only 2-3 mV with SNS. Spontaneous miniature endplate potentials (MEPPs) could be detected in muscles with SNS at day 11 and stimulation of nerve proximal to crush elicited action potentials in muscle fibers indicating substantial regneration at the crush site. HRP was seen streaming through the crush region and could be detected in motoneurons of the ipsilateral ventral horn of the lumbar spinal cord. In hibernating animals with SNS or LNS, a 10-16 mV depolarization of surface fibers was seen in the EDL muscle at day 30. After 15-20 days for SNS and 30-40 days for LNS, muscle action potentials could be evoked only through stimulation of distal nerve segment. However, muscles studied at 60-120 days post crush showed no evidence of MEPPs or muscle twitch in response to stimulation of nerve proximal or distal to the crush. In the hibernating squirrels, retrograde transport of HRP at 30 and 120 days showed absence of reaction product in ipsilateral ventral horn cells in the lumbar cord. The extensive traumatic operation in hibernating animals kept them awake 15-22 days (body temp 35-37° C). The rate of regeneration of nerve after nerve crush in such animals was similar to that seen in nonhibernating squirrels. The evidence presented here indicates that although degeneration of the distal nerve stump is prolonged during hibernation, no reinnervation of muscle occurred as revealed by electrophysiological recordings. (Supported by NIH grants NS12063 and HL17800.)

246.13 THE SENSITIVITY OF REGENERATING NERVE ENDINGS TO CALCIUM AND 3,4 DIAMINOPYRIDINE. T.M. Argentieri*, J.J. McArdle, S. Laxminarayan* and L. Michelson*. Depts. of Pharmacology and Scientific Data Processing, New Jersey Medical School, UMDNJ, Newark, NJ 07103.

The effect of calcium (Ca) and 3,4-diaminopyridine (3,4 DAP) on the binomial parameters describing quantal release of acetylcholine was examined at reinnervating neuromuscular junctions (NMJ) in the extensor digitorum longus muscle (EDL) of female Wistar rats. Animals were sacrificed 11 to 14 days following nerve crush and the EDL with nerve attached was removed. Transmitter release was then evaluated by the direct method. End-plate potentials (EPPS) and miniature end-plate potentials were recorded in crush fiber preparations correcting all amplitudes for membrane capacitance and EPPS for non-linear summation (Martin, J. Theor. Biol. 59:179, 1976). Several statistically significant ($p<0.05$) observations were made at reinnervating NMJ'S: 1) the mean quantal content (m) was reduced from a control value of 35.10 $\pm$ 5.93 (mean $\pm$ SEM) to 17.73 $\pm$ 1.33, 2) the probability of release was lowered from a control value of 0.98 $\pm$ 0.01 to 0.90 $\pm$ 0.05 and 3) the number of release sites, as reflected by the n-statistic, was reduced from a control value of 42.34 $\pm$ 1.81 to 25.06 $\pm$ 1.53. The sensitivity of the transmitter release mechanism to extracellular Ca concentrations (2.0 mM to 0.6 mM) was next examined. The slope of m versus Ca concentration from reinnervating NMJ'S was significantly lowered from a control value of 16.34 $\pm$ 3.90 (mean $\pm$ 95% CL) to 10.00 $\pm$ 2.37. This suggests a decrease in the responsiveness of the regenerating release process to extracellular Ca. To further examine this ionic dependancy, muscles were superfused with 3,4 DAP (5-25 µM). Following this, the probability of release at reinnervating NMJ'S was significantly increased from 0.90 $\pm$ 0.05 to 1.04 $\pm$ 0.02. There was also an increase in the percentage of fibers with EPPS, suggesting a "turning on" of nerve terminals not previously releasing detectable amounts of transmitter. These data suggest that during nerve regeneration, there exists a defect in the Ca dependent mechanism of transmitter release and that this defect may be partially overcome by 3,4 DAP. Supported by NIH grant NS 11055-09.

246.14 PHARMACOLOGICAL FACILITATION OF RECOVERY AFTER SCIATIC NERVE DAMAGE IN THE RAT. S. Mazzella*, D. Calzolaio*, R.F. Marotta*, H. Weiner and E.L. Gardner. Dept. of Psychiatry, Montefiore Hospital and Albert Einstein College of Medicine, Bronx, New York.

Experimental work has shown a number of biologically active compounds to be capable of accelerating the rate of functional recovery after peripheral nerve damage. These agents include dibutyryl-cyclic-AMP (Pinchichero et al., Science 182:724,1973), ACTH (Strand & Kung, Peptides 1:135, 1980) and triiodothyronine (Cockett & Kiernan, Exp. Neurol. 39:389, 1973). In addition, a most intriguing study was published by Wolf in 1940 (J. Nerv. Ment Dis. 92:614) indicating that chronic administration of prostigmine increased measured restitution of function in sciatic nerves damaged by alcohol injection. Apart from these in vivo studies, other investigators have had an in vitro focus. We have studied several different agents alone and in combination to explore possible therapeutic interventions and to set a groundwork for the study of possible mechanisms involved in pharmacological facilitation of recovery.

Individually housed, ad lib fed and watered, male Wistar rats, weighing 280 gm., were subject to hemostat crushing (2mm.; 10 sec.) of each sciatic nerve above the bifurcation of the peroneal and tibialis nerves. The crush was verified by electrical stimulation and upon emerging from anesthesia complete paralysis of the hindlimbs was noted. Rats were randomly assigned to one of five treatment groups: (1) saline control, n=38; (2) neostigmine .25 mg/kg/day, n=34; (3) aminophylline 15 mg/kg/day, n=32; (4) neostigmine-aminophylline, n=24; (5) neostigmine-adrenaline .25-.25 mg/kg/day, n=24. Animals were rated daily by two observers, blind as to treatment, for ability to walk, movement of the knee, foot placement, toe spread, and pain withdrawal, before each daily drug treatment.

For all categories studied the medicated animals reached the criterion of complete recovery faster than controls. For example, the mean number of days to complete recovery of walking were: saline 19.2; neostig. 15.6; aminophyll. 17.6; neo.-amino. 15.8; and neo.-adren. 15.4. For foot placement: saline 18.7; neostig. 17.3; aminophyll. 15.2; neo.-amino. 14.5 and neo.-adren. 15.2. ALL ANOVA and Tukey comparisons were significant at least at the .05 level. These data clearly show pharmacological facilitation in a clinically relevant model by drugs currently in general use.

247.1 MEMBRANE STRUCTURE DURING POSTNATAL SYNAPTOGENESIS IN CEREBELLAR GLOMERULI. J.J. Halperin, L.A. Weinstein*, D.M.D. Landis. Dept. of Neurology, Massachusetts General Hospital, Boston, MA. 02114

We have examined the formation of synaptic junctions between mossy fiber axons and granule cell dendrites in the granular layer of developing mouse cerebellar cortex. In mature animals, the synaptic junction is about 200nm in diameter, and has prominent electron-dense fuzz lining the postsynaptic membrane. In freeze-fractured preparations, there is a distinct aggregate of particles associated with the extracellular half of the fractured postsynaptic membrane, clustered on an indentation into the dendritic contour coextensive with the widened synaptic cleft. In developing animals, most of the synaptic junctions evident in thin sections are larger than in adults; the proportion of small, mature synaptic junctions increases with age. With freeze-fracture techniques, we recognized three sets of structures in the developing granule cell dendrites: there were loose aggregates of particles on the extracellular half of the membrane not obviously associated with any changes in membrane contour; there were aggregates of particles associated with indentations of the dendrites which were less densely packed than the particles at mature junctions and which covered a larger area that mature synaptic junctions; and there were indentations, usually larger in area than those at mature synaptic junctions, which had no associated particle aggregates. The developing and mature synaptic junctions were easily distinguished from puncta adherentes in thin sections and in freeze-fractured preparations.

It seems likely that particles loosely aggregated on the indented regions of the dendritic contour correspond to the larger synaptic junctions visualized in thin sections of immature animals, and that the particles become progressively more tightly packed as the junctional area becomes smaller. The nature of the particle aggregate not associated with an indentation is less certain; perhaps these are proteins inserted into the membrane, able to aggregate, but also able to translocate to sites where junctions will form. The presence of indentations without associated particles may indicate that the synaptic cleft can form before the aggregate assembles. (supported by NS 00353, 15573, and 06638)

247.2 SPATIO-TEMPORAL OCCURRENCE OF COATED VESICLES DURING PURKINJE CELL DEVELOPMENT AND SYNAPTOGENESIS. S. Chen and D. E. Hillman. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016.

Previous studies have correlated the appearance of coated vesicles with receptor-mediated endocytosis, membrane recycling and exocytosis for various types of secretory granules. In the nervous system, recent studies show a marked increase in the number of coated vesicles within axons and dendrites during reactive and developmental synaptogenesis. This study reports a marked increase in the number of coated vesicles in the soma and dendrites of Purkinje cells which corresponds to the time of synaptogenesis. In the soma, coated vesicles appeared as blebs with a continuous channel to Golgi cisternae or to the smooth endoplasmic reticulum. Distinct coating of Golgi membranes was found on the secretory side of the complex. The second common site of coated vesicles was on or near the plasma membrane of the soma, dendrites and spines. A channel was traced in serial sections from the surface of the dendrite into coated vesicles and to a small constricted connection with the ER. Less frequently, coated vesicles were located within the dendrite and occurred as bleb-like extensions of the ER. Quantitation for the spatio-temporal occurrence of coated vesicles in Purkinje cell somata and dendrites revealed high densities of coated vesicles before synaptogenesis. This level was 7 times higher in dendrites of 4-day-old rats than for adults and 4 times higher in the soma. A drop in the density of coated vesicles in both the somata and dendrites occurred by 8 days and was followed by marked resurgence in their density at 10 days. Subsequently, the number declined in both from about 12 to 21 days, corresponding to parallel fiber synaptogenesis. The intermittent drop in number of coated vesicles at about 8 days may have been due to the sudden onset of climbing fiber synaptogenesis and/or changes in the volume of somata and dendrites. The continuity of coated vesicles with the Golgi apparatus, ER and plasma membrane indicates that an ER channel occurs between the Golgi apparatus and the plasma membrane of somata and dendrites. Our study supports suggestions that macromolecules of synaptic junctions are formed in the Golgi apparatus and are transported along the membrane of the endoplasmic reticulum as bud-like vesicles to sites of potential afferent input. Supported by USPH grants NS13742 & HD10934.

247.3 DEVELOPMENT OF ACETYLCHOLINE RECEPTOR CLUSTERS AT NERVE-MUSCLE CONTACTS IN XENOPUS CULTURES. H. Kuromi* and Y. Kidokoro. The Salk Institute, San Diego, CA. 92037.

Muscle cells in culture have discrete clusters of acetylcholine receptors (AChRs) along with a widespread distribution of the receptors. When innervation occurs, a region of high receptor density develops at the site of nerve-muscle contact. To date, the mechanism of receptor accumulation at the site of nerve-muscle contatct is unknown. Nor is it known what happes to receptor clusters outside of the nerve contact region. In the present study, we examined the sequential changes in the distribution of AChRs on identified muscle cells co-cultured with neural tube cells.

Nerve and muscle cultures were prepared from embryos of Xenopus laevis. Muscle cells were stained for AChRs with tetramethylrhodamine-conjugated α-bungarotoxin. Distribution of fluorescence staining on the muscle cell was recorded on a video tape recorder through an image intensifier and subsequently analyzed by using a digital video processor. With the use of an image intensifier, successive observations could be performed on an identified muscle cell without bleaching of fluroescence. Initially, small fluorescent speckels (<1μm) emerged from the background at the sites of nerve-muscle contact. They increased in number and fused to form larger clusters. Sometimes large clusters of AChRs associated with nerve elongated or separated into several smaller clusters along the nerve. Then the distance between the smaller clusters increased. As a result, the area of high AChR density increased along the path of nerve-muscle contact. When the nerve disappeared or died, the majority of the receptor clusters associated with the nerve disaggregated into smaller clusters or speckles and finally disappeared into the background. This observation indicates that nerve-induced accumulation of AChRs is reversible at the early stages. At the non-junctional region, in some cases, large fluorescent clusters or speckles became faint or disappeared during the process of AChR accumulation along the path of nerve-muscle contact. So far, we have not obtained firm evidence that the cluster moves in the muscle membrane as a whole. By studying the sequential process of AChR accumulation, we hope to obtain information elucidating the underlying molecular mechanism for this developmental process.

247.4 IDENTIFICATION OF FACTORS MEDIATING SYNAPSE FORMATION. D.M. Michaelson*, N.R. Ways* and B.G. Wallace, Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

To examine mechanisms by which nerve and muscle communicate to establish synaptic connections, we have developed a simple assay for monitoring the initial steps of synapse formation using established techniques for culturing chick neurons and myotubes. Muscle cells are dissociated from 12 day chick embryos with trypsin and plated on 24 well collagen-coated tissue culture plates. Explants of 10 day chick embryo ciliary ganglia are added to the cultures after the myoblasts have fused to form multinucleated myotubes. Within 24 hours myotubes become functionally innervated. The extent of synapse formation is determined by observing the cultures on an inverted phase microscope and counting myofiber twitches. Uninnervated myotubes rarely twitched; fibers contacted by neurons contracted in response to synaptic potentials. Twitching was abolished by curare or α-bungarotoxin. The frequency of nerve-evoked twitches was enhanced by 10^{-4} M 4-aminopyridine (4-AP). Intracellular recordings from myotubes showed that 4-AP increased the frequency and amplitude of spontaneously occurring synaptic potentials without significantly changing their time course.

To identify factors mediating synapse formation mice were immunized either with dissociated iris muscles from 10 day chick embryos or with extracts of nerve-muscle cocultures. Spleen cells from immunized mice were fused with SP2/0 myeloma cells and hybrid clones screened for their ability to block synapse formation. 130 clones from 2 fusions have been examined. The supernatant from one clone was found to reduce synapse formation without i) preventing neurite outgrowth, ii) blocking muscle contractions evoked by direct application of carbachol, or iii) blocking transmission at established synapses. Studies are in progress to characterize this antigen as well as to generate additional clones. In this way we aim to identify signals required to initiate the formation of neuromuscular junctions.

This research was supported by BRSG grant RR5353 and PHS grant NS16440.

247.5 EFFECT OF CYCLOHEXIMIDE ON THE FORMATION OF APPARENT PRESYNAPTIC ELEMENTS. Richard W. Burry, Department of Anatomy, College of Medicine, The Ohio State University, Columbus, Ohio 43210.

Axons of cultured neurons will grow onto polylysine coated sepharose beads and form apparent presynaptic elements with the bead in the position of the postsynaptic element. In this model system for studying synaptogenesis it was found that apparent presynaptic elements form within 3 hours and show a steady increase in the number of synaptic vesicles for at least 9 days. This model offers an excellent system for investigating the need for protein synthesis by the soma for the growth of the axon and the formation of the presynaptic-like element.

Cultures of rat cerebellums were exposed to various concentrations of cycloheximide for 1 hour in the presence of ^{3}H-leucine. Scintillation counts showed that 25 µg per ml of cycloheximide inhibited 99% of the protein synthesis. Light microscopic autoradiography confirmed that neuronal cell protein synthesis was inhibited. Trypan Blue exclusion showed that neuronal cell death began between 24 and 48 hours and by 72 hours most neurons were dead. Experiments with cultures allowed to recover after cycloheximide treatment, showed that most neurons survived a 48 hour treatment, but that few survived a 72 hour treatment.

Cultures were examined for the presence of apparent presynaptic elements after treatment with 25 µg per ml of cycloheximide and polylysine coated beads. In cultures exposed to the drug for a total of 13 and 25 hours, normal numbers of apparent presynaptic elements were seen. At 36 hours the number of apparent presynaptic elements began to decrease and this trend continued through 48 hours. The reduction in the number of apparent presynaptic elements, and the occurence of neuronal cell death prior to 48 hours was associated with the massive death non-neuronal cells at this time. Although the number of elements decreased by more than half at 48 hours, the mean area per element and the mean density of synaptic vesicles in the element did not differ from untreated controls.

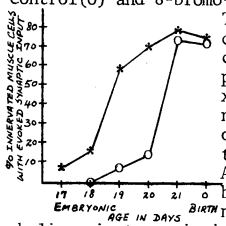
The lack of change in the morphology of the apparent presynaptic element through 48 hours supports the idea that inhibition of neuronal protein synthesis for 48 hours does not affect the growing axon and its formation of apparent presynaptic elements. The decrease in the number of apparent presynaptic elements by 48 hours was probably due to the death of neurons which in turn was caused by the inhibition of protein synthesis. Supported by NIH Grant NS-15894.

247.6 MATURATION OF CHOLINERGIC TRANSMISSION IN RAT RETINAL NEURONS IN CULTURE: REGULATION BY 3',5'-ADENOSINE MONOPHOSPHATE (cAMP). H.H. Yeh and D.G. Puro, Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, Maryland 20205.

An important aspect of synaptogenesis is the emergence of effective synaptic transmission. We are using a cell culture system to examine electrophysiologically the regulation of the step in which a presynaptic neuron becomes capable of transferring information across a newly-formed synapse. Here, we report that analogs of cAMP accelerate this developmental step in cultured cholinergic neurons of the rat retina.

Trypsin-dissociated retinal cells from embryonic day 17 to newborn rats were cocultured for 1 day with rat striated muscle cultures. Functional retina-muscle synapses form rapidly, as determined by the presence of spontaneous synaptic potentials detected by intracellular recordings from muscle cells. Early in the functional maturation of these synapses, neurotransmitter release cannot be evoked by excitatory stimulation. This non-transmitting period is followed by a transmitting phase, in which iontophoretic applications of glutamate can evoke acetylcholine release from cholinergic retinal neurons.

In our culture system, 8-bromo-cAMP or dibutyryl cAMP (1mM) precociously induced the onset of stimulus-bound cholinergic transmission, as depicted by the developmental time course for the control(o) and 8-bromo-cAMP-treated(*) cultures in the inset.



This accelerating effect was dependent on concentration (half-maximal=50 µM 8-bromo-cAMP) and also could be mimicked by the phosphodiesterase inhibitor, isobutylmethylxanthine(1mM). Specificity of the cyclic nucleotide effect was suggested by a failure of 8-bromo-cGMP to accelerate the onset of this phase of cholinergic transmission. Additional experiments showed that when 8-bromo-cAMP was pressure ejected near retinal neurons in control cultures, stimulus-bound cholinergic transmission was induced within minutes. Similar results were obtained using iontophoretically applied dopamine, a putative neurotransmitter that increases cAMP levels in rat retina.

These data provide evidence for a cAMP-mediated regulation of synaptic activity during retinal development. We postulate that certain transmitters (e.g., dopamine) may work through the cAMP system to facilitate the induction of effective neurotransmission. A related hypothesis to be tested is that certain of the mechanisms which regulate neurotransmission at developing synapses also have a physiological role in adult retinal function. Our culture system should be a useful model for examining these issues concerning synaptic function.

247.7 EFFECT OF VISUAL CORTEX ABLATION ON SYNAPTIC VESICLE ANTIGEN IN THE DEVELOPING RABBIT SUPERIOR COLLICULUS. K.F.Greif and A.S.Kelly*. Dept. of Physiology, Univ. of Calif., School of Medicine, San Francisco, CA 94143.

The effects of removal of visual cortex input on synapse number in the superior colliculus (SC) has been studied in both adult and developing rabbits using a monoclonal antibody which binds to a 65 kdal protein localized to the outer surface of synaptic vesicle plasma membrane of all neuron classes examined thus far (Matthew et al, J. cell biol, 91: 257, 1981). Synapse number was indirectly assessed by measuring the amount of synaptic vesicle antigen (SV Ag) present in whole tissue homogenates. Inhibition radioimmune assays were used to quantitate the amount of SV Ag present in homogenates of rabbit SC after unilateral lesion of primary visual cortex (decorticate SC). The unoperated side of the animal was assayed as an internal control, and the ratio of SV Ag in decorticate and normal SC determined. Cortical lesion in five adult animals resulted in an average decrease of 35% in SV Ag levels in SC after survival times of seven or 14 days (t-test, $p<.005$). Six rabbits lesioned at birth, before corticotectal fibers have arrived in the SC (Kelly and Schwartz, Soc. Neurosci. Abstr., 6: 485, 1980), showed an average increase of 14% in SV Ag levels in SC when assayed at 14, 35 or 150 days postnatal (t-test, $p<.05$).

Preliminary results from rabbits lesioned at different ages during the first postnatal month, when complete corticotectal input to the SC is normally established, suggest that the observed "overproduction" of SV Ag (and presumably of synapses) after neonatal removal of the visual cortex is normally prevented by competition between retinal and cortical input to the SC.

This research was supported by NIH Grant 1-R01-NS16702 to ASK and NIH Training Grant 2-404945-24171-3. Additional support was provided by NSF Grant BNS-8100342 to L.F. Reichardt.

247.8 STUDIES ON THE CHANGES IN THE PATTERN OF SYNTHESIS OF AXONAL PROTEINS DURING SYNAPTOGENESIS. P. Sonderegger*, M.C. Fishman, M. Bokoum*, P.A. Pudimat*, H.C. Bauer, and P.G. Nelson. Lab. of Developmental Neurobiology, National Institutes of Health, Bethesda, MD 20205.

The study of synapse formation in vitro between two or more populations of cultured cells has the advantages of better accessibility of the cells, control of the environmental conditions and lower complexity. For biochemical studies such as the search for changes in the protein pattern which may take place in the presynaptic or postsynaptic cell during synapse formation and consecutive synapse stabilization events, the cells involved in the formation of a synapse should be accessible for independent stimulation and biochemical analysis.

This technical requirement for the study of synapse formation in vitro can be fulfilled by the three chamber system devised by B.Campenot (PNAS, 74:4516-4519,(1977)). In our experiment, dissociated cells from embryonic chicken dorsal root ganglia were plated in the central compartment of the culture chamber and their axons allowed to grow under the barrier through a thin film of medium into the two side compartments. Presumptive postsynaptic cells, i.e. spinal cord cells, were plated in the two side chambers and synapse formation studied electrophysiologically. The presynaptic cells with axons growing from the center chamber to the side chambers were stimulated extra-cellularly with platinum electrodes by bipolar voltage pulses across the barrier and showed thresholds between 0.3-0.8 V. Elicited postsynaptic potentials were registered by intra-cellular recording from the post-synaptice cells in the side chambers. The proteins synthesized by the presynaptic cells were labeled with [^{35}S] methionine applied to the cell bodies in the central compartment. The axonal proteins collected from the side compartments were examined by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). It was found that leakage of radioactive label from the side chambers was negligible in that no contamination of axonal proteins with proteins synthesized by the cells of the side chambers occured.

Comparison of the 2D-PAGE of DRG axons alone or in the presence of presumptive postsynaptic cells revealed a high degree of similarity. However, a very acidic protein with a molecular weight around 75 K Dalton was eliminated from the presynaptic cells' axonal protein patterns, when presumptive postsynaptic cells were present. Whether the elimination of this protein in the presynaptic axon is specifically due to the establishment of synaptic connections or is induced by the change in the cellular environment (neuronal and/or non neuronal) remains to be elucidated by work currently under way.

247.9 MODIFICATION OF AN AXONALLY TRANSPORTED PROTEIN IN TOAD RETINOTECTAL TERMINALS. Pate Skene, Mark Willard, and John A. Freeman. Dept. of Anatomy, Vanderbilt Univ., Nashville, TN 37232; and Dept. of Anatomy-Neurobiology, Washington Univ., St. Louis, MO 63110.

Stabilization or elimination of a newly-formed synapse seems to depend on usage of the synaptic connection and on competition with other synapses terminating on the same cell; so there must be some property of functional synaptic terminals which distinguishes them from non-synaptic or non-functional synaptic axon terminals. We report here that mature retinal terminals differ from non-synaptic or pharmacologically blocked terminals in the post-translational modification of an 18 kilodalton membrane protein.

We injected ^{35}S-methionine into the posterior eye chambers of toads (Bufo marinus) and used two-dimensional gel electrophoresis and fluorography to analyze the most rapidly moving group of labeled proteins transported into retinal axons in the optic nerve, optic tract, and optic tectum. In optic nerve or tract membrane fractions taken 14 hours after injection, an 18K protein showed more rapid migration in non-equilibrium pH gradient electrophoresis (NEPHGE) than did a similar-sized protein in tectum samples from the same animals, suggesting that the 18K protein is post-translationally modified when it reaches synaptic terminals. The "tectal" 18K protein is not the product of local protein synthesis in the tectum, since direct application of ^{35}S-methionine to the tectum does not label that protein.

We next crushed optic nerves at the chiasm immediately before injecting the radiolabel, then allowed 14 hours for labeled proteins to accumulate in the crushed axon ends; the 18K protein from these nerves showed NEPHGE migration similar to that of the 18K protein from normal nerves. Preliminary experiments also indicate that the 18K protein is not converted to the "tectal" form in tecta in which retinotectal transmission has been blocked post-synaptically with α-bungarotoxin for 4-8 days.

Our results suggest that post-translational modification of the 18K protein requires functional contact with a post-synaptic cell; 18K modification therefore might be involved in stabilization of synapses.

Supported by NIH grants EY01117-10 (JAF) and EY0 2682 (MW) and the Jordan Fellowship of the National Spinal Cord Injury Association (PS).

247.10 THE FINE STRUCTURE OF EMBRYONIC NEOCORTEX TRANSPLANTED INTO ADULT MOUSE NEOCORTEX. Leslie M. Smith and Ford F. Ebner. Brown University, Providence, RI 02912.

We are using neural transplantation to study factors that control elongation of axons and formation of synapses in the adult cerebral cortex. A first step in the analysis has been to describe the normal morphology of the transplants, especially their interface with the host tissue, and to determine whether fibers from the corpus callosum will grow into and form synapses with the transplanted neurons.

In 40 adult mice (BALB/cj), rectangular solids (.5x.5x4mm) of embryonic cortex (E-17) were successfully implanted in the sensory-motor area and allowed to survive for 30 days. In 10 of the 40 animals the corpus callosum was surgically sectioned after the 30 day survival period, 2 days before perfusion. All animals were perfused for EM, and tissue blocks were cut orthogonal to the long axis of the transplant and processed for electron microscopy.

The transplants are clearly visible at the time of blocking; they are about 1mm in diameter and extend from layer I to the white matter. The surface of the host brain is neither sunken nor bulging out and after 30 days there are no signs of necrosis or hemorrhage along the interface between donor and host tissue. One um sections through the transplants show a well vascularized oval of donor tissue embedded neatly in the host brain. The transplants contain three clearly different subareas; a hilus of myelinated and unmyelinated axons, a large zone of loosely arrayed cells and a small cell-free zone along the border opposite the white matter.

The transplants contain a well developed synaptic neuropil with both RA and FS types of synapses present. The ratio of FS to RA contacts is larger in the transplant than in host tissue. Both pyramidal and stellate cell types can be identified. Dendritic spines are found throughout the transplant, but occur in highest density in the cell-free zone. Neocortical transplants from these E-17 donors show a paucity of glial cells and glial processes. Some astrocytic processes are found along the interface region where they are indistinguishable from host astrocytes. The fine structure of host and donor tissue are distinguishable at the interface region by subtle criteria, such as the size and density of myelinated axons, but there are no pathological or unusual features, such as glial scarring or macrophages, that mark the line of transition.

Section of the corpus callosum produces moderate degeneration throughout the expected layers of the host cortex. Degenerated axons and terminals are found in the transplants, although clearly fewer in number than in the host cortex. These results indicate that the implants can be innervated to some extent by commissural fibers. (Supported by NINCDS grant #13031)

247.11 THE DEVELOPMENT OF CHOLINERGIC MARKERS IN NORMAL AND TRANSPLANTED MOUSE NEOCORTEX. Christine F. Hohmann* and Ford F. Ebner (SPON: C. Lent). Brown University, Providence, RI 02912.

In rats, the development of cholinergic markers in neocortex closely parallels the development of synapses (Coyle and Yamamura, 1976; Johnson and Armstrong-James, 1970). We are interested in whether there is a causal relationship between these two developmental processes. Transplantation of developing cortex into adult cortex of genetically identical animals offers a system in which we can manipulate the cholinergic input during synaptogenesis. We report here baseline observations on the development of cholinergic markers in normal and transplanted mouse (BALB/cJ) neocortex.

ChAT activity was measured according to the method of McCaman and Hunt as modified by Fonnum. ChAT activity is not present in detectable levels in normal mouse cortex until the end of the first postnatal week. Enzyme activity reaches 50% of adult levels during the fourth postnatal week and rapidly achieves adult levels after that time.

AChE histochemistry was performed according to the method of Hardy et al. AChE reaction product becomes visible in the normal cerebral cortex of BALB/cJ mice on postnatal day 1. Only layer VI and the underlying white matter stain at this early age. Bands of AChE staining gradually become visible in layers V-IV and I during the following days to reach adult levels after the third postnatal week.

Explants of developing cortex (.5x.5x4mm) from days E-18 to PND 2 donors were transplanted into adult host sensory-motor cortex and allowed to survive for periods up to 60 days. The transplants were contained entirely within the donor cortex, where they occupied a roughly circular zone about 1mm in cross-section.

AChE histochemistry showed that transplants allowed to survive over 2 weeks always contain detectable AChE reaction product. The density of staining never achieved host levels at any survival time. The reaction product was frequently densest in a circular pattern around the edge of the transplant. On close inspection, this staining overlapped a cell-free zone that always occupied one border of the transplant. Elsewhere in the transplant the staining pattern was rather diffuse and showed little lamination. Postnatal donor tissue showed a denser staining pattern at any given survival time than did tissue from prenatal donors. These results suggest that the initial state of development of the cholinergic system in the donor at the time of transplantation may influence the later development of cholinergic markers in the transplant. (Supported by NINCDS grant #13031.)

247.12 THE VASCULARIZATION OF NEOCORTICAL TRANSPLANTS. Perry Busalacchi* and Ford Ebner. Brown University, Providence, RI 02912

Embryonic cortex continues to differentiate when placed in the sensory-motor cortex of adult mice. One important factor for continued survival of transplant cells is the rate and degree of vascularization. A well-developed network of blood vessels is always present after the transplant has survived for 30 days. We are now examining the time course of vascularization using India ink perfusion after survival periods of increasing duration.

Embryonic cortex from gestational day 14 to 18 was implanted in adult host mice (BALB/cJ) with a capillary tube. The implants were placed in sensory-motor cortex and allowed to survive for periods from 3 to 60 days. After the survival period, the animals were anesthetized and perfused with 4% paraformaldehyde followed by 10ml of India ink. The brains were removed and immersed in the perfusion fluid with 30% sucrose added. Sections were cut at 50um on a freezing microtome, mounted, and Nissl-stained.

By 9 days after transplantation, blood vessels in the transplant fill with India ink as well as they fill in the host cortex. Prior to 7 days, the vascular pattern always appears incomplete and blood vessels usually fail to fill at all. The density of blood vessels in the transplants appears stable after 14 day survivals. Even after 30-60 day survival period, the density of blood vessels in the transplant never achieves that of the host. After long-term survivals, there is frequently a network of vessels running along the host side of the interface between donor and host tissue. This dense network is typically seen only on one side of the transplant. Preliminary measurements of the length of blood vessels per unit volume of tissue (um/um^3:n=10 cases) indicates that the length density of blood vessels is only 60% that of the host cortex. When the transplant is cut in cross-section, the percentage is always lower than when the transplant is cut longitudinally, so there may be some inherent orientation to the vascular pattern. Computer reconstructions of the 3-dimensional array of vessels in the transplant and surrounding host tissue are being carried out to visualize the internal geometry of the vascular patterns, and to determine whether there is a preferential ingrowth from the molecular layer or the white matter side of the transplants.
(Supported by NINCDS grant #13031.)

247.13 SYNAPSE FORMATION AND MATURATION IN THE VISUAL CORTEX OF THE RAT. M.E. Blue and J.G. Parnavelas. Dept. of Cell Biology, Univ. of Texas Health Science Center, Dallas, TX 75235.

The formation and maturation of synapses in the rat visual cortex were examined by electron microscopy at postnatal days 0,2,4,6,8,14,16,20,28 and 90. A quallitative and quantitative examination was performed which involved the observation of coronal sections through the visual cortex and the analysis of photographic montages of 50 µm wide strips of tissue that extended from the pia to the white matter. For the quantitative analysis, the total density of synapses (synapses/100 μm^2 neuropil) and the densities of type I and type II synapses were calculated at all ages examined. Histograms of synaptic density as a function of depth were also prepared.

At birth, synapses containing well-defined membrane specializations and several vesicles were present, particularly in the subplate region. Immature forms, identified by the presence of synaptic specializations and an occasional vesicle, were also recognized. The maturation of synapses was a continuous process that occurred during the first four weeks of life. At day 28, synapses appeared qualitatively similar to those observed in adult rats.

Synapses were confined to the subplate region at day 0 and by day 6 they were distributed throughout the cortex but prevailed in layers I and V. An adult-like distribution of synapses, with the highest densities present in the upper layers, was attained by day 14.

The majority (90%) of cortical synapses were of the type I variety. At birth, the densities of type I (0.16 synapses/100 μm^2) and type II (0.02 synapses/100 μm^2) synapses were low. The time course of development was different for the two synapse types. Type I synapses were formed rapidly in the first four weeks of life and particularly between days 2 and 16 (0.18 to 6.32 synapses/100 μm^2); they then declined from days 28 to 90 (7.34 to 6.41 synapses/100 μm^2). The number of type II synapses did not increase significantly until day 6. A continuous increase was seen in their density during the second week and a dramatic increase from days 14 to 16 (0.15 to 0.42 synapse/100 μm^2). Subsequently, their frequency declined significantly to adult values (0.23 synapse/100 μm^2). The time of rapid growth of type II synapses coincided with a period of hypertrophy of non-pyramidal cell perikarya. It may be significant that these events occurred shortly after eye-opening.

This work was supported by USPHS grant EY02964.

247.14 DEVELOPMENT AND SYNAPTOGENESIS IN FETAL OLFACTORY CORTEX. H. Newman* and L. E. Westrum (SPON: A. B. Harris). Depts. of Neurological Surgery and Biological Structure, Univ. of Washington, Seattle, WA 98195.

Although the newborn prepyriform cortex (area 51) is characterized by significant numbers of mature synaptic structures (Westrum, L.E. and Miller, E., Neurosci. Abstr., 5, 1979), little is known about the situation in the fetus. This study intends to characterize various developmental aspects of this region at selected prenatal ages both at cellular and ultrastructural levels. Rat fetuses aged E16-E21 (birth at E21.5) from time-mated Sprague-Dawley dams are being used to study the sequences of initiation and formation of the different types of synapse as well as maturation of other neuronal and non-neuronal elements. The earlier ages (E16-18) are characterized by a large, well-formed lateral olfactory tract (LOT), a very cellular layer I, and a wide layer II with neurons in various stages of maturation. By E20-21, layer I is more distinct, has fewer scattered cell bodies and layer II becomes clearly defined with numerous mature neurons. E21 is similar to the newborn in these features. Synaptic contacts are obvious by E17 but generally they are very immature in nature. The forms of these contacts are variable and include those situations where either and pre- or postsynaptic specialization may be the more developmentally advanced of the two. In addition, presumed non-innervated postsynaptic sites occur. Although some synapses are seen in the upper part of layer I, their frequency appears greater near the layer II cell bodies. These deeper synapses often are more mature than the superficial ones. Totally mature contacts are uncommon at E16-18 but increase in numbers in older fetuses. Actual counts of synapses are underway to determine precise populations and distributions. These observations show a comparatively early appearance of a few primitive synaptic structures in a relatively unorganized layer I of olfactory cortex at a time when the main afferent olfactory pathway (LOT) is disproportionately large. Also the apparently greater numbers of mature contacts deeper in layer I suggests an early gradient of synaptogenesis. (Supported in part by N.I.H. Grants No. NS 09678, 17111-01S1 and DE 04942. LEW is also an affiliate of the CDMRC.)

247.15 EARLY EVENTS IN SYNAPSE FORMATION ON SYMPATHETIC NEURONS OF THE FROG. Lawrence M. Marshall* (SPON: D. Sinicropi). Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27514.

The early development of the innervation of sympathetic neurons was investigated in the ninth and tenth paravertebral ganglia of Rana pipiens. Preganglionic nerve fibers were stained by anterograde transport of horseradish peroxidase and examined by light and electron microscopy at various stages of larval development before and during metamorphosis. At each stage, intracellular recordings were made from principal neurons during stimulation of the preganglionic nerve trunk.

During limb bud stages (stages I-V; Taylor & Kollros, Anat. Rec. 94:7, 1946), the sympathetic chain is a long mass of small, uninnervated cells not clustered in ganglia. At the developmental stage at which only the hindlimbs are exposed (stage XVII), discrete ganglia are present along the sympathetic chain. Preganglionic axons first grow into the ninth and tenth ganglia at about stage XXI, when the tadpole has forelimbs and hindlimbs, and the tail is beginning to degenerate. Five to six days later (stage XXIII), many of the neurons are contacted by a single preganglionic axon and exhibit infrequent spontaneous excitatory potentials and subthreshold responses to nerve stimulation. By the end of metamorphosis (stage XXV), 8-10 days later, the neurons receive synaptic inputs from 2 to 4 axons, some of which are capable of triggering an action potential.

These findings establish that, in Rana, synapse formation by preganglionic axons begins after the formation of the sympathetic chain ganglia during the very late stages of larval development. (Supported by NIH grant NS 17203).

247.16 EFFECTS OF SYMPATHETIC SYNAPTOGENESIS IN HIPPOCAMPUS ON EXTRACELLULAR SINGLE UNIT ACTIVITY. D. J. Barker and A. J. Howard*. Dept. of Physiology, Texas College of Osteopathic Medicine and North Texas State University, Fort Worth, TX 76107.

Normally, terminals of noradrenergic sympathetic fibers arising from the superior cervical ganglion are found only in the cerebral vasculature, pineal and habenula. Following ablation of the rat septal region (which also interrupts noradrenergic innervation of hippocampus from locus coeruleus), sympathetic terminals in the hippocampal vasculature sprout collaterals which grow into the granule cell layer of the dentate gyrus initially and the entire hippocampus subsequently. Recent evidence suggests that there are behavioral changes related to this sympathetic ingrowth. The present study is part of an attempt to determine if the growth of sympathetic fibers into the dentate region can be demonstrated to be functional electrophysiologically. The purpose of this study was to examine spontaneous activity rate and pattern of firing in dentate granule cells of septal animals with and without sympathetic ingrowth. It has been suggested that endogenous hippocampal norepinephrine (normally from locus coeruleus) acts to maintain low firing rates. Thus, it was expected that firing rates in rats with septal lesions and sympathetic ingrowth would be comparable with rates in normal controls. Septal rats with no ingrowth were expected to have higher baseline firing rates.

Extracellular single units were recorded from the dentate granule cell region in 3 groups of rats anesthetized with chloral hydrate: normal rats (N=22), rats with septal lesions (N=54) and rats with septal lesions and superior cervical ganglion removed (N=27). Recording was done 3 to 6 weeks post-surgery and responses analyzed by computer. Lesions, recording sites and ingrowth were verified histologically. Three distinct response patterns in spontaneously active cells were clearly evident in all 3 groups: non-bursting cells (40%), regular bursting cells (35%) and irregular bursting cells (25%). Firing rates in rats with septal lesions and no ingrowth (MDN=6.9/sec) were significantly higher than in controls (MDN=4.2/sec) while rates in rats with septal lesions and ingrowth (MDN=5/sec) were not significantly different from control rats. Analysis of rates among different response types indicated that this increase was due to nonbursting cells. In addition, among septal rats with no ingrowth, mean interval for irregular bursting cells (5.8 ms) was significantly shorter than in controls (10.3 ms). Mean interval in septal rats with ingrowth (7.5 ms) did not differ from controls. These results suggest that sympathetic ingrowth in the hippocampus may have some functional capability in a non-specific modulatory manner. Subsequent experiments will examine the effects of sympathetic stimulation on granule cell unit activity. Supported by AOA Grant 81-11-019.

247.17 EFFECT OF DOPAMINERGIC DEAFFERENTATION ON THE POSTNATAL DEVELOPMENT OF THE RAT NEOSTRIATUM. Martin R. Krigman, Elizabeth G. Bendeich,* and George R. Breese University of North Carolina, Chapel Hill, NC 27514

Neostriatal synaptogenesis is in the rat a postnatal phenomenon which is modulated by genetic and epigenetic factors. In general, the ultimate organization of the synaptic pattern is dependent upon the appropriate temporal appearance of extrinsic and intrinsic synaptic input. The effect of disrupting the dopaminergic afferent input on neostriatal synaptogenesis was investigated by neurochemical, histochemical and morphometric analyses.

Long Evans neonatal rats were treated on the day of birth 60 min after desipramine (20 mg/kg) with an intracisternal injection of 6-hydroxydopamine (100 ug) or a saline (Smith et al., J.P.E.T., 185:609, 1973). Pups were allowed to develop and were sacrificed for this study when 45 days of age. Dopamine concentration in the striatum was less than 1% of control values. Electronmicroscopic studies were limited to a predefined region of the neostriatum. Fine structural histochemical methods (Falk, B., Acta Physiol Scan 56:Suppl:197, 1962) demonstrated that less than 0.1% of the neostriatal boutons contained small granular vesicles in the 6-hydroxydopamine-treated rats compared to the 8 - 12% observed in the controls. Morphometric analyses revealed a significant reduction in the volume density of the neuropil but no changes in either the myelin volume density or numerical density of neurons. There was also a reduction in both the numerical density of synapses and the synapses per neuron in the treated rats. When analyzed in terms of synaptic connection, based upon axo-spinous, axo-dendritic, or axo-somal type, there were no discernable changes in the proportion of synaptic type between the control and treated groups. However, when the synapses were analyzed utilizing the criteria proposed by Hassler (J. Neurol. Sci. 36:187, 1978) there was a marked reduction of the nigral-striatal bouton in the 6-hydroxydopamine-treated rats. Other bouton forms (Hassler, 1978) exhibited no appreciable reduction; there was, however, a uniform increase in the proportion of other terminals.

The reduction in the numerical density of synapses and synapses per neuron, is greater than the expected 10 to 15% that could be ascribed to loss of nigral dopamine afferents. The basis for this degree of synaptic loss is not apparent. There was on the other hand no evidence of any unique compensatory proliferation of other extrinsic or intrinsic connecting synaptic systems observed after destruction of afferent dopamine-containing neurons.

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248.1 REGENERATION OF EFFECTIVE BUT ABERRANTLY DISTRIBUTED SYNAPSES BY SENSORY NEURONS IN THE LEECH CNS, E.R. Macagno[1], S.A. DeRiemer[2] and K.J. Muller[3]. Columbia University[1], New York, NY 10027, Yale University[2], New Haven, CT 06511, and Carnegie Institution of Washington[3], Baltimore, MD 21210.

Neurons that regenerate synapses with their normal targets could do so either by growing back to their original locations or by growing abnormally and finding the normal targets at ectopic locations in the neuropil. We have studied this problem by examining the regeneration of leech touch sensory neurons (T cells) one day to four years after we cut or crushed connectives between ganglia. T cells arborize in the segmental ganglion containing their cell bodies and in the adjacent anterior and posterior ganglia. Separate injection of Lucifer Yellow (LY) and horseradish peroxidase (HRP) into two ipsilateral T cells within either the same or adjacent ganglia reveals a striking coincidence in the positions of branches and varicosities of the two cells, which we have used as an index for the degree to which regenerating axons reconstruct their normal arborizations in adjacent ganglia. The degree of functional regeneration was assayed by testing for restoration of synaptic transmission from each T cell to the S interneuron in the target ganglion and, in a few cases, the reinnervation of the skin by sensory axons. After electrical recording a T cell was injected with HRP, which filled regenerated processes in target ganglia within two days. A homologous T cell in the target ganglion was then filled with LY and the tissue processed for simultaneous viewing of HRP and LY in whole mount (Macagno et al., Brain Res. 217:143-149, 1981). The degree and pattern of regeneration were evaluated by means of computer-assisted analysis of the distribution and number of branches, varicosities and contacts between homologous touch cells.

The analysis of more than 50 cases of T cell regeneration reveals that: (a) Within a few days to weeks of the time of the crush or cut an extensive amount of sprouting occurs at the site of the lesion. In many cases numerous sprouts cross the site and grow towards the target ganglion, but generally only one grows into the ganglion; evidently the others are retracted; (b) In a small number (less than 10%) of cuts or crushes, continuity (as judged by HRP diffusion) with a distal stump is re-established within a few days, a phenomenon similar to that reported for several systems (e.g., Hoy, Bittner and Kennedy, Science 156:251-252, 1967); (c) Among those cells that regenerate and form functional connections, the degree to which regenerated patterns of arborization resemble the normal pattern is quite variable and the spatial distribution of regenerated branches and synapses can be markedly abnormal. Thus, regeneration of normal functional connections can occur despite morphologically abnormal regeneration.

248.2 Adult lampreys can recover from complete spinal cord transection. Joseph Ayers, Scott Currie*, James Kinch* and Wanessa Pereira* (SPON: Helen Mahut). Department of Biology and Marine Science Institute, Northeastern University, Boston, MA 02115.

Larval sea lampreys (Petromyzon) can recover competency for several behaviors following CNS lesions as severe as complete transection of the spinal cord. In contrast, it is generally believed that this capability is lost following the adult metamorphosis which occurs following the 6th or 7th year of larval life. To test this hypothesis, we have transected anadromous lampreys at the level of the ninth myotomal segment posterior to the gill arch at all 6 morphological stages of transformation and in completely metamorphosed adults. In contrast to the previous beliefs, we have found that both transforming and transformed adults can recover competency for several behaviors.

There is a major behavioral difference between adults and larvae following transection. In contrast to larvae, adults exhibit persistent "attached undulations" which may occur for several months following transection. Quantitative behavioral analysis of these "undulations" demonstrates that they are quantitatively similar in their dynamics to the movements produced by bath application of 2mM d-Glutamate in specimens with their spinal cords surgically exposed to the bath ("fictive swimming"), but different with regard to cycle period, intersegmental delay and wave curvature from normal adult swimming.

In addition to swimming, we have scored recovery for four other behaviors including forward and backward crawling, burrowing and aversive withdrawal. In general, the recovery times for all of these behaviors are slower than those observed in larvae and partial recovery is more frequently observed. Quantitative analysis of films of transectee behaviors reveals similar deficits to those observed in larvae: a reduction in the amplitude of movements compensated by an increase in flexion wave frequency and conduction velocity.

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248.3 **ELECTROPHYSIOLOGICAL EVIDENCE OF REGENERATION OF LAMPREY SPINAL NEURONS.** M.E. Selzer and H.S. Yin. Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Spinal cords of sea lamprey larvae were transected at the level of the cloaca. After at least 70 days recovery, giant interneurons (GIs) located caudal to the transection scar were impaled with microelectrodes and activated by rostral cord stimulation. By correlating the antidromic and orthodromic responses with the distances of cell bodies and cord stimulating electrodes from the transection scar, the distance of regeneration and the degree of synaptic reconnection were evaluated.

GIs, which normally project rostrally, often could be antidromically activated by stimulating the spinal cord within 1-5 mm rostral to the scar. (However, one GI was antidromically activated from as far as 13.5 mm rostral to the scar.) Regeneration of more caudally situated rostrally projecting axons which made en passant synapses onto GIs was also demonstrated by recording monosynaptic EPSPs with the same stimulus locations.

Stimulation of the cord further than 5 mm rostral to the scar usually evoked monosynaptic EPSPs in GIs located within 5 mm caudal to the scar. This suggested regeneration of caudally projecting axons up to 5 mm. The EPSP latencies suggested proximal axon conduction velocities of 0.5 to 0.65 m/s. This is much slower than expected for the giant reticulospinal axons (RAs). It is consistent with morphologic evidence that caudally transected RAs generally did not grow past the scar, even though most RAs transected closer to their cell bodies did regenerate.

When stimulating electrode and impaled GIs were both more than 5 mm from the scar, only EPSPs of variable latency, i.e. polysynaptic EPSPs, were recorded from GIs.

Conduction velocities of regenerated neurites usually were slower than their parent axons. For example, in the GI which regenerated 13.5 mm, the conduction velocity of the rostral neurite was 0.8 m/sec, while that of the caudal parent axon was 1.8 m/sec. This correlates with the reduced diameters of regenerating neurites.

We conclude that both rostrally and caudally projecting axons of transected larval lamprey spinal cord can regenerate, generally up to 5 mm, but rarely more. NIH grants NS14837, NS14257, RR05415.

248.4 CHANGING CONDUCTION PROPERTIES OF EARTHWORM GIANT FIBERS DURING REGENERATION. E.P.Vining and C.D.Drewes*. Dept. of Zoology, Iowa State Univ., Ames, IA 50011

We have investigated regeneration of giant nerve fibers in the earthworm, Eisenia foetida, following complete transection of the ventral nerve cord. Conduction properties of lesioned and subsequently regenerated giant fibers were tested in vivo using a grid of electrodes etched onto a printed circuit board. This permits multi-channel recording of medial (MGF) and lateral (LGF) giant fiber spikes through the skin of intact, unanesthetized earthworms in response to tactile stimulation anterior or posterior to the lesion. Animals were tested at 4 hr intervals from 16 until 56 hr after lesioning.

Restoration of spike coupling across the lesion occurred as early as 20 hr after transection in 5 of 20 animals. With ventral nerve cord cuts made at segment 25, recovery of MGF coupling across the lesion occurred significantly earlier than LGF coupling (MGF mean = 38 hr $\pm$ 10 SD, LGF mean = 48 hr $\pm$ 16 SD, $\alpha = 0.05$, n = 20). However, with cuts made at segment 75-80, there was no significant difference between the recovery of MGF and LGF coupling (MGF mean = 40 hr $\pm$ 11 SD, LGF mean = 46 hr $\pm$ 14 SD, n = 20). The intial coupling of MGF and LGF spikes in most animals was reliable in either direction across the lesion. In a few animals, however, initial coupling was labile with occasional failure of spike conduction across the lesion in one or both directions, but within 4 hr conduction across the lesion became more reliable and usually bidirectional.

The conduction time for initially coupled MGF spikes across the lesion (2 mm between electrode pairs) was 2.4 ms $\pm$ 0.2 SE (n = 5), or considerably greater than for LGF spikes (1.1 ms $\pm$ 0.1 SE, n = 5). These values are considerably greater than conduction times over a 2 mm distance adjacent to the lesion (MGF = 0.29 ms $\pm$ 0.01 SE, n = 5; LGF = 0.43 ms $\pm$ 0.06 SE, n = 5), the latter values being identical to the conduction times of normal, unlesioned giant fibers. By 24 hr after initial coupling mean conduction times across the lesion had decreased substantially (MGF = 0.8 ms $\pm$ 0.1 SE, n = 5; LGF = 0.7 ms $\pm$ 0.1 SE, n = 5).

These results indicate that within 20-48 hr following nerve cord transection there is 1) no significant alteration of the conduction properties in the giant nerve fibers adjacent to the lesion, and 2) a rapid return to near normal (i.e., rapid, reliable, and bidirectional) conduction across the lesion. These studies demonstrate the significant capacity for rapid recovery of conduction in earthworm giant nerve fibers and the utility of this system for in vivo resolution, hour by hour, of the changing properties of regenerating nerve fibers.

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248.5 GIANT NERVE FIBER FUNCTION IN REGENERATING TAIL SEGMENTS OF EARTHWORMS. C. D. Drewes* and B. L. Marty* (SPON: R. G. Sherman). Dept. of Zoology, Iowa State Univ., Ames, IA 50011.

Classical experiments in regeneration have demonstrated that earthworms are among the most highly organized animals capable of complete regeneration of lost body segments. In this study we have examined conduction properties and patterns of growth of newly organized giant nerve fiber systems within regenerated tail segments of the earthworm, Eisenia foetida. By day 7 after removal of 36-44 posterior segments from adult worms, small and undifferentiated tail buds were formed. Tactile stimulation of these buds initiated lateral giant fiber (LGF) spiking activity in older segments just anterior to the bud. By day 9, segmental boundaries were discernible in the bud, with subsequent bud growth involving increases in segmental dimensions but little if any increase in the total number of bud segments (mean = 23 segments $\pm$ 6 S.D. n = 8). By day 14 the ventral nerve cord was visible through the ventral body wall of the bud and all-or-none LGF spikes could be detected within the bud in response to tactile stimulation applied to the bud or to older segments adjacent to the bud. By day 14, LGF conduction velocity was 0.8 m/s, or nearly identical to the velocity of just formed LGF systems in late embryonic worms. During subsequent bud growth LGF velocity increased to 2.4 m/s by day 28 and 3.2 m/s by day 42. These rates of conduction velocity increase are essentially identical to those seen during postembryonic growth in normal worms, suggesting that processes underlying giant nerve fiber formation and growth in regenerating tail segments recapitulate those occurring during normal pre- and post-embryonic development.

Tail regeneration is not necessarily restricted to conditions of posterior segment removal (termed homomorphic tail regeneration), but can occasionally occur following removal of anterior segments (termed heteromorphic tail regeneration). Both heteromorphic and homomorphic tails were found to be exclusively subserved by the LGF sensory field. Medial giant fiber (MGF) activity was also detected in regenerated tails, but only in response to stimulation within the MGF sensory field of original segments. These results indicate that the reformation of LGF sensory field in tail segments occurs independently of the location of regeneration along the body axis.

Supported by the Terrestrial Biological Monitoring Program of the U.S. Environmental Protection Agency.

248.6 A HORSERADISH PEROXIDASE STUDY OF PERIPHERAL NERVE REINNERVATION INTO THE SPINAL CORD. K. K. Messenger* and R. E. Kingsley, Department of Neuroscience, South Bend Center for Medical Education, Indiana University, South Bend, IN 46556.

Previous work has demonstrated that peripheral axons can reinnervate the spinal cord under appropriate circumstances (1,2). Other attempts at reinnervation have failed (3). Only one class of grafts has been shown to be physiologically functional (1). Most of these graft types have not been studied with modern anatomical methods. The purpose of this study was to verify and to determine the extent of reinnervation of peripheral neurons into the adult mammalian spinal cord using various grafting techniques. For this study we chose horseradish peroxidase (HRP) as the axonal marker.

Adult cats were grafted by one of the following methods: 1) the proximal stump of the L7 ventral root was anastomosed to the proximal stump of the L6 dorsal root (VR-DR); 2) the L6 dorsal root was sectioned 2 cm. from the spinal cord and then rejoined (DR-DR); 3) the central stump of the L6 ventral root was grafted into a slit in the lateral funiculus of the spinal cord (VR-LF); 4) the distal stump of a sectioned DR was grafted into a slit in the lateral funiculus of the spinal cord (DR-LF). Normal dorsal roots were used as controls. At various time intervals ranging from 2-78 months the grafted nerves were retrieved and sectioned 2 to 5 mm from the neuroaxis. The proximal end of the graft was placed in a 50% solution of HRP for 24 to 36 hours before the cat was sacrificed. The enzyme marker was visualized in 40 uM sections using the method of Adams (4).

Labeled neurons were found in the dorsal funiculus and the dorsal root entry zone of the VR-DR graft and the DR-DR graft at all time intervals tested. These were more lightly labeled than controls. Darkly labeled neurons could be followed into the spinal cord at the entry site of the DR-LF or VR-LF grafts. The fibers of these grafts appeared to encounter a scar tissue barrier and either stopped growing or grew into whorls. In these cases, scar tissue overcame the graft site and dislodged the axons from the spinal cord in 3 to 4 months.

From this study we conclude that reinnervation of a peripheral nerve into the spinal cord over a pre-existing pathway is not limited by peripheral nerve growth or morphology. These nerves can reinnervate the spinal cord and remain viable for up to 78 months. Entry via artificial pathways is ultimately aborted.

1) C. D. Barnes & N. Worrall, J. Neurophysol. 31:689 (1968)
2) K. Ikeda & J.B. Campbell, Exp. Neurol. 30:379 (1971)
3) D. L. Kimmel & E. K. Moyer, J. Comp. Neurol. 87:289 (1947)
4) J. C. Adams, Neuroscience 2: 141 (1977)

248.7 AXONAL REGENERATION FROM LONG SPINAL TRACTS. P.M. Richardson, V.M. Issa* and A.J. Aguayo. Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4.

Peripheral nerve grafting, shown in previous experiments to enhance the growth of some spinal and cerebral axons, was used here to investigate axonal regeneration in ascending and descending spinal tracts. One lateral column of the spinal cord was cut at either the low cervical level (11 rats) or midthoracic level (7 rats) and one end of a sciatic nerve graft was stitched to the meninges at the site of injury. The grafts were approximately 1.5cm long and the distal end was joined to the lower trunk of the brachial plexus or left free on the thoracic paravertebral muscles. Three to five months after operation, the grafts were re-exposed and injected with horseradish peroxidase (HRP). Two days later, the rats were sacrificed and multiple sections through the brainstem and thoracolumbar spinal cord were processed with tetramethyl benzidine. Neurons in the red nucleus (mean = 5 neurons), ventrolateral pontine tegmentum ($\bar{n}$ = 7), vestibular nucleus ($\bar{n}$ = 6), and raphe and medullary reticular formation ($\bar{n}$ = 19) were labelled retrogradely by injections into cervical grafts. Only 2 neurons were found in the brainstem of the 7 rats with thoracic grafts. Thus, axons in each of the 4 major bulbospinal pathways grew from the cervical region into peripheral nerve segments but few such axons were shown to regenerate after injury in the midthoracic region. In either experimental preparation, HRP-containing neurons were found in distal spinal segments, particularly in Clarke's column, the ipsilateral nucleus proprius and the intermediate grey matter. The mean number of labelled neurons in a 1.0cm length at the thoracolumbar junction was 64 after cervical grafting and 100 after thoracic grafting. For these regenerating fibres in ascending spinal tracts, the distance from cell body to site of injury was as great as 5cm.

Long ascending and descending axons in the lateral columns of the rat spinal cord possess at least some ability to regenerate. In some instances, the regenerative behaviour of bulbospinal neurons is influenced by the level at which their axons are injured as well as by the neuroglial environment at the axonal tip.

248.8 AXON GROWTH FROM RAT BRAIN STEM NEURONS INTO PNS GRAFTS. M.Munz* and A. Aguayo. Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4.

The capacity of certain neurons in the adult mammalian brain and spinal cord to grow along peripheral nerve grafts has been recently documented (Nature 284:264, 1980; Nature 296:150,1982; Science 214: 931, 1981). In the present study we investigate further this capability at all three levels of the rat brain stem.

In 225-300 g Sprague Dawley rats, an autologous, 3.5 cm long segment of sciatic nerve was introduced into the lower brain stem and directed rostrally into the medulla or pons. The other end of the graft was placed into the dorso-lateral region of the cervical spinal cord. The neuraxis between the two types of graft insertion was left intact; the span of these PNS bridges being extraspinal. By 2-3½ months after transplantation the graft contained many regenerated fibers. The origin of some of these axons was traced retrogradely with horseradish peroxidase (HRP, Sigma VI, 20%) applied to the cut ends of the graft approximately 2½ cms from the brain stem.

Numerous labelled neurons were found in most nuclei of the medulla oblongata, pons and mid brain as well as in the gray matter of the spinal cord. The distribution of the labelled cells varied in relation to the position of the grafts; the area of greatest density being within a few millimeters of its tip. The number of labelled neurons in some of these animals was greater than that observed in other graft experiments where only a small lesion was made into the lateral medulla (Science 214:931, 1981). These findings suggest that the site and extent of the grafting procedure strongly influences the distribution and number of nerve cells recruited into growth. Because axons from the labelled neurons appear to have grown for a distance of at least 2 cms the results obtained provide additional evidence for a more widespread potential for extensive elongation in neurons of the central nervous system.

248.9 GRADUAL LOSS AND PARTIAL RECOVERY OF A PROXIMAL MOTOR REFLEX FOLLOWING SPINAL TRANSECTION IN LAMPREYS (PETROMYZON MARINUS).
S. N. Currie* and J. L. Ayers . Dept. of Human Physiology, Univ. of California, Davis, Ca* and Dept. of Biology, Northeastern Univ., Boston, Ma.

Lampreys normally maintain an upright posture while swimming. Orientation relative to gravity is sensed and adjusted via a well developed vestibular-reflex system, responding to lateral rotation of the labyrinths with movement of the neck and fins appropriate to righting (Rovainen, J. Neurophys., 42:745, 1979). In order to quantify righting capacity in the following studies, swimming animals (all larvae) were observed for 100 seconds, noting the relative time spent dorsal-side up. Results were ther converted to percentile scores. Preliminary experiments, aimed at localizing those areas of the body crucial to posture control, involved scoring animals before and after the denervation of discrete regions in the head, neck and trunk. Results show that a small, specific portion of the neck (about 10 muscle segments), centered just behind the last gill, is alone sufficient to maintain normal equilibrium, while its loss results in major disability. The fins, and a larger portion of the rostral trunk, may be utilized normally to some extent, but are not required.

Following spinal transection at mid-body, far distal to this critical region, animals exhibit a progressive loss of vestibular control. Kept at 23°C, the deficit becomes most pronounced after 7-8 days, and is never completely reversed, though partial recovery will usually occur after many weeks. The rate at which recovery proceeds appears to be directly proportional to body length. Whether this is a function of length itself or of larval age however, is unclear.

A possible neural correlate of the behavioral loss is presently being investigated in the paired Müller cells, M_3 and I_1. Both neurons are known to receive vestibular input and are excited by downward rotation of the contralateral labyrinth (Rovainen, 1979). Furthermore, both give rise to large spinal axons extending from the brain to caudal fin, and when stimulated, produce strong EPSPs in neck motorneurons (Rovainen,1979). The axotomy of these cells which occurs with spinal transection has been shown by Fishman (1975) to result in marked chromatolysis and dendritic resorption. Work is presently under way to determine whether vestibular input to these cells is reduced as a result of chromatoytic changes, and if so, how the time course of this effect corresponds to that of behavioral disfunction.

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248.10 TRANSPLANTATION OF FETAL SPINAL CORD TISSUE TO INJURED SPINAL CORD IN NEONATAL AND ADULT RATS.
B.S. Bregman and P.J. Reier, Dept. of Anatomy, Univ. Maryland, Sch. of Med., Baltimore, MD 21201

Several attempts have been made to obtain anatomical repair in the injured spinal cord using transplants of fetal spinal cord tissue. These efforts, however, have yielded variable results regarding the survival of the implants and their axonal integration with the host spinal cord. The aim of the current study was to determine whether implants of fetal spinal cord survive and form axonal connections with the host spinal cord in animals lesioned neonatally or as adults. Neonates were used since: (1) tissue reactions to injury which impede axonal outgrowth in the adult, are less severe in the immature spinal cord, and (2) a population of late-developing axons (e.g. corticospinal (CST)) escape direct damage. After incomplete spinal cord lesions, immature CST fibers take an aberrant route through intact CNS but do not regenerate across a lesion site in the absence of a suitable substrate. Spinal cord lesions were made at T_6 in neonatal (<72 hr) and adult rats. The lesions included complete transections and partial lesions which spared some of the most ventral fibers. Donor tissue was prelabeled with ^{3}H-thymidine by intraperitoneal injection into the mother at E12, E13, and/or E14. Fetuses were obtained at E14 and 1-2mm^3 segments of spinal cord were implanted into the lesion site. After 1-4 months, HRP was injected into the spinal cord (N=8) in order to determine whether anatomical connections were formed between the host and implant tissues. Specimens were processed for light (LM) and electron (EM) microscopic examination. Spinal cord implants were identified in 10 of 14 (71%) of the adult and 17 of 23 (73%) of the neonatal operates. In both groups implants contained mature neurons, predominantly of small and medium diameter surrounded by a neuropil of myelinated and nonmyelinated fibers. LM and EM showed extensive areas of apposition between host and implant without an intervening glial barrier. Cellular bridges containing axons extended between the implant and host tissues. HRP injections into the lumbar cord (20-25 mm caudal to the implant) retrogradely labeled neurons and anterogradely labeled fibers within the implant. In addition, retrograde labeling of CST neurons occurred in the neonatal operates; preliminary anterograde tracing experiments indicated that some of the CST fibers grew through the implant. These results indicate that implants of fetal spinal cord survived and formed anatomical connections with the host CNS and that neurons within the implant projected their axons for long distances within the host spinal cord. These results suggest that the implant may serve either as a potential relay for supraspinal input, or a bridge for growing fibers as suggested by the growth of the CST in the neonate.

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248.11 FAILURE OF TECTAL EFFERENT NEURONS TO REGENERATE IN THE FROG (RANA PIPIENS) FOLLOWING DIENCEPHALIC OR ISTHMAL HEMISECTIONS.
M.J. Lyon and D.J. Stelzner, Anatomy Dept., SUNY Upstate Medical Center, Syracuse, NY 13210. Supported by NIH Grant NS 14096.

The left tectum of 19 frogs was injected with ^{3}H-proline (0.004-0.05µl; 10-50µCi/µl; housed at 20-22°C). The animals were killed 24 hrs later, and the brains processed for autoradiography. This protocol was used for all ^{3}H-proline injected animals; all animals where horseradish peroxidase (HRP) was applied were killed after 24 hrs and reacted for HRP activity with cobalt enhanced diaminobenzidine (Adams, J.C., Neurosci., 2:131, 1977; Malmgren, L.T. and Y. Olsson, Brain Res. 148:279, 1978). The majority of the results agreed with Lazar (Acta Biol. Acad. Sci. Hung. 20:171, 1979).

After a brainstem hemisection just caudal to the isthmal nucleus, 20 frogs, at different time periods (1-20 wks) received an ipsilateral tectal injection of ^{3}H-proline. While all other tectal efferent projections appeared normal, there was no detectable ipsilateral descending projection beyond the site of transection. Some axons may have regenerated around the lesion site to descend with the contralateral projection. If this was the case, they failed to return, in detectable numbers, to their normal sites.

The regenerative capacity of ascending tectal efferents was examined using anterograde HRP and autoradiographic techniques. At intervals (1-30 wks, N=43) following a diencephalic hemisection, either an ipsilateral tectal injection of ^{3}H-proline was made or a pledget soaked with 50% HRP 5% dimethyl sulfoxide in 0.6% saline was positioned over several small holes placed in the ipsilateral tectum. Since the major termination sites of the ascending projections are the ipsi- and contralateral medial thalamic neuropil (MTN; this projection crosses in the postchiasmic commussure) any label in these regions post-hemisection would be a strong indication the regeneration had occurred. In all but two of the animals examined (12 wks) there was no evidence of regeneration back to the contralateral MTN. The dorsal ipsilateral MTN did show increased activity. While some of this increase could be due to spread of label from the injection site, the HRP studies indicated that some axons do regenerate over and around the lesion site. These axons in the MTN had varicosites characteristic of synaptic endings. The vast majority of axons terminate at the proximal edge of the lesion site. These results are similar to those found in the mammalian central nervous system after a lesion. Due to the negative results found in all animals up to 9 wks post lesion, it is likely that the lesions in the two animals showing label in the contralateral MTN were incomplete.

248.12 RETROGRADE DEGENERATION OF CUT CENTRAL AXONS IS INHIBITED BY APPLIED ELECTRIC CURRENT.
E. Roederer*, N.H. Goldberg*and M.J. Cohen (SPON: J. Rosenbaum). Dep't. of Biology, Yale Univ., New Haven, Connecticut 06511.

The spinal cord of the lamprey Petromyzon marinus has giant reticulospinal neurons which regenerate following transection. By 100 days after lesion, new synapses have formed distal to the lesion and swimming has returned. In the mammalian CNS, there is a partial retrograde degeneration or "die-back" of severed axons in the proximal spinal cord stump that may extend for several mm rostrally from the lesion. In the lamprey spinal cord, where regeneration does occur, we show in this study that axonal die-back also occurs and that it preceeds regeneration. The die-back during the first 5 days post-transection is significantly reduced by application of 10 µA DC current across the site of transection with the cathode distal to the lesion. Reversing the polarity of the applied current (cathode proximal to the lesion) increases the extent of axonal die-back relative to the sham treated controls.

Following spinal transection, saline-filled wick electrodes were implanted in the body musculature on either side of the lesion and the animals were divided into 3 treatment groups: (i) a control group with implanted electrodes but no current passed across the lesion, (ii) a group in which a 10 µA DC current was passed across the transection with the cathode distal to the lesion, (iii) a group in which a 10 µA DC current was delivered with the cathode proximal. At the end of 5 days, the brain and attached proximal spinal cord segment were removed and several axons in each preparation were injected with the flourescent dye, Lucifer Yellow. The extent of axonal die-back in the severed giant axons was determined in the filled fibers by measuring the distance of the axon end from the site of lesion. The mean distances of axonal die-back for the 3 treatment groups were as follows: (i) controls, 1750 µm ± 45 SEM (36 axons), (ii) cathode distal, 740 µm ± 33 SEM (37 axons), (iii) cathode proximal, 2820 µm ± 60 SEM (34 axons). The results were analyzed using the Wilcoxon Rank Sum Test and the differences between the treatment groups were found to be significant: cathode distal vs. cathode proximal electrode placement, $p<0.01$; cathode distal vs. controls, $p<0.01$; cathode proximal vs. controls, $p<0.05$.

An endogenous injury current composed predominantly of sodium and calcium ions is known to enter the cut end of the spinal cord for several days after transection (Borgens, Jaffe and Cohen, PNAS, 77:1209, 1980). We propose that axonal die-back following transection is caused primarily by entry of these cations into the cut end of the axon due to the injury current. The applied electric current modulates the endogenous injury current by either counteracting or enhancing it and thus determines the extent of subsequent die-back. An applied current that bucks the endogenous injury current reduces axonal die-back, while a current that enhances the injury current increases die-back. (Supported by N.I.H. Spinal Trauma Center Grant 2P50 NS 10174-09).

249.1 DECREASED THYROXINE LEVELS IN ARTIFICIALLY REARED RAT PUPS. C. Stamper, F. Petracca*, V. Houghton* and J. Diaz. Dept. of Psych., University of Washington, Seattle, WA 98195.

The importance of thyroid hormones in normal brain development has been well established. Hypothyroid rat pups have been shown to have a decrease in the size of cortical neurons, decreased cortical weight, a decrease in RNA/DNA ratio, a decrease in phospholipids and cholesterol concentration (Ford and Cramer, Thyroid Hormones and Brain Development, 1977. Although TRH, TSH, T4 and T3 are all present in mammalian milk, their functional significance to brain development in the offspring is not clear (Kodolvsky, Life Sciences, vol 26, 1980).

The purpose of the present study was to assess T4 levels and brain growth in rat pups reared away from their mothers, and receiving: 1) a replacement formula without any amounts of mother's milk, and 2) this same formula supplemented with TRH.

Four day old female Long-Evans hooded rat pups were matched by weight and assigned to one of 3 groups: 1) normally reared animals (NR) who remained with their mother, n=8, or 2) artificially reared animals (AR), n=8, or 3) artificially reared animals with 12ug TRH per day added to the milk formula, (AR-TRH), n=8. The animals received chronic intragastric infusions of a milk formula according to a technique previously described (Diaz, et al., Physiology and Behavior, vol 27 ,1982) They were sacrificed on day 18 and blood was collected for serum T4 analysis. Their brains were removed, dissected and weighed.

Even though there were no significant differences in body weight on day 4, by day 18, the animals in the NR group weighed significantly more than the animals in either the AR or the AR-TRH group ($p<.05$). Whole brain, brain stem and cerebellar weights were also significantly larger for the animals in the NR group than for the animals in either the AR or AR-TRH group ($p<.05$). The serum T4 levels were significantly higher in the NR group than in the AR or AR-TRH group ($p<.05$). Post hoc Scheffe tests revealed no differences in any measure including T4 levels between the AR and the AR-TRH groups ($p<.05$).

These data demonstrate that when animals are reared away from their mothers and without access to her milk, they have a diminished level of thyroid functioning on day 18. In previous studies in our laboratory, delayed appearance of developmental milestones has not been noted in artificially reared animals. The normal appearance of developmental milestones may not be entirely mediated by thyroid hormone levels. When a rat pup is not given access to mother's milk during the weanling period, thyroxine levels are appreciably lowered by day 18. The specific role of thyroid elements in mother's milk remains unclear.

249.2 ULTRASTRUCTURAL CHARACTERISTICS OF CELLS OF THE MESENCEPHALIC NUCLEUS OF V DURING DEVELOPMENT IN NORMAL AND HYPOTHYROID RATS. Y. Narayanan* and C.H. Narayanan. Dept. of Anat., LSU Sch. of Med., New Orleans, LA. 70119.

In the present study we have compared the ultrastructural characteristics of neurons of the mesencephalic nucleus V of between normal and hypothyroid rats in fetal and neonatal stages. Timed pregnant rats (Holtzman) were raised on a goitrogenic diet consisting of 0.5% and 0.3% propylthiouracil (PTU) mixed with powdered rat chow beginning from seven days of pregnancy. Control animals were raised on powdered rat chow without PTU. All animals had ad lib access to food and water. Midbrain and rostral hindbrain regions were dissected out in selected cases, according to routine procedures, and the region of the mesencephalic nucleus of V was processed for electron microscopy.

Cells of the mesencephalic nucleus are readily identifiable on the basis of their large size, and appear as a narrow crescent-shaped cluster of cells at the periphery of the central gray matter of the aqueduct, extending from the posterior commissure to the level of the motor nucleus of V. Major changes in morphogenesis of this nuclear center are observed during a three-week period after birth. Cytoplasmic reorganization, neurite formation, synaptogenesis and myelination occur during this period in normal development. A preliminary survey of our material shows that differences in cellular morphology between control and treated groups at the ultrastructural level are recognizable from day ten postnatally. In the control group of animals, the nucleoli have well differentiated Pars granulosa and Pars fibrosa. Granular endoplasmic reticulum and groups of free ribosomes are well developed and form organized Nissl bodies. Numerous mitochondria are present with compact cristae. The cytoplasm also contains tubular and filamentous profiles in abundance. Numerous profiles of a granular reticulum making up the Golgi complex are observed with small vesicles budding off from the 'forming' face. The neurons of the hypothyroid group, in contrast, show a reduction in neurotubules and filaments. The nucleoli are condensed into a crystalline mass. The Nissl material is widely dispersed and lacks the organization seen in control animals at corresponding stages of development. The cristae of the mitochondria are dilated and the Golgi complex is poorly represented. The results of our study are consistent with the view that thyroid hormones play an important role in selected aspects of neuronal differentiation and development, and in the regulation of metabolic homeostasis. Supported by the National Institutes of Health-National Institute of Child Health and Human Development. HD 12064.

249.3 ADRENALECTOMY-INDUCED BRAIN SIZE INCREASES: ACCELERATED MATURATION OR PERMANENT CHANGE? J. A. Devenport & L. D. Devenport. University of Oklahoma, Norman, Oklahoma 73019

Although we have previously demonstrated that adrenalectomy increases brain size (Devenport, Behav. Neural Biol., 1979, 27, 218-221) and that its effect is reversed by replacement of corticosterone (Devenport, et al. Neurosci. Abstr., 1980, 6, 382) the permanency of this effect has not been investigated. The present study was designed to determine if the removal of adrenal glands simply hastens the maturation of the CNS or if it exacts an enduring change in structure which would persist well beyond full maturity.

Subjects were male Sprague-Dawley derived rats that were adrenalectomized (ADX) or sham-operated (SHAM) at 25 days of age. They were randomly selected from 17 different litters. Following surgery, they were given continuous access to 1% saline and lab chow. Individually housed at 30 days, they received no further handling until sacrifice.

At day 65 about half of each group (n=14 ADXs and n=15 SHAMs) were sacrificed and measurements were taken on fore- and hindbrain weight, forebrain dimensions, body weight and length. The remaining ADXs (n=12) and SHAMs (n=19) were sacrificed at 145 days of age and the same measures were taken.

The brains of ADXs were significantly larger than those of SHAMs at both ages, demonstrating again the adrenalectomy-induced brain growth effect. The absolute rate of brain growth diminished across time in both groups; however, and most importantly, the relative rate of divergence in brain weight between the two groups increased as a function of age, suggesting that the brain becomes increasingly sensitive to adrenocortical hormones as age progresses. The maturation hypothesis can be unambiguously rejected.

249.4 THYROXINE-INDUCED MATURATION OF THE EGL AND THE MOLECULAR LAYER IN THE FROG CEREBELLUM. K.F. Hauser* and A.G. Gona. Dept. of Anatomy, UMDNJ-New Jersey Medical School, Newark, N.J. 07103.

This study examined changes in the external granular layer (EGL) and the molecular layer following thyroxine (T_4)-induced metamorphosis in premetamorphic bullfrog tadpoles (Rana catesbeiana), and compared these to events seen in spontaneous metamorphosis. T_4 was administered intraperitoneally on alternate days, 0.25ug/g body wt. during the first week and 0.38ug/g body wt. thereafter. The tadpoles were maintained at 20±1°C. The cerebellum was examined by using the Golgi-Kopsch method and TEM after 1, 2 and 3 weeks of T_4 treatment and compared with premetamorphic controls. Degenerating cells were observed in the EGL and outer molecular layer at all time periods studied following T_4 treatment. This usually consisted of a few scattered cells, the number and location being variable. Degenerating cells were seen infrequently in spontaneously metamorphosing tadpoles. Therefore, T_4-induced metamorphosis may accelerate a normally occurring process, although cell death might reflect T_4 toxicity. In addition, immature stellate cells were found in the outer molecular layer near the EGL after 2 weeks of T_4 treatment. Stellate cells were distinguished from migrating granule cells by the presence of multiple processes (some ending in growth cones) oriented parallel or at oblique angles to the pial surface; synaptic contacts on the soma and differentiating processes; and dispersed chromatin in nuclei with 1 or 2 invaginations. Stellate cell differentiation during spontaneous metamorphosis was similar, although the degree of maturation varied between individual neurons suggesting a protracted period of stellate cell generation during spontaneous metamorphosis. This suggests that in the frog, as in other species, stellate cell precursors are formed from the EGL, and that their appearance is dependent on thyroid hormone (in frogs). Lastly, Purkinje cell dendritic growth was apparent after 2 weeks of treatment and followed a pattern similar to that initially occurring during spontaneous metamorphosis, i.e., rapid dendritic growth (elongation) with little branching (Uray and Gona, JCN, 185:237). Many of the growing tips of Purkinje cell dendrites could be seen ending in growth cones with one or more filopodia. These processes often grew towards the pia, terminating in the molecular layer just beneath the EGL. Developing dendritic spines frequently contacted parallel fiber synapses. The qualitative similarities between T_4-induced and spontaneous metamorphosis with respect to Purkinje cell dendritic development further suggest that cerebellar maturation in the frog is largely a thyroid-dependent phenomenon.

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249.5 DEXAMETHASONE-INDUCED ALTERATIONS IN HIPPOCAMPAL DEVELOPMENT. K.J. Anderson*, S.W. Scheff and S.T. DeKosky. Departments of Anatomy and Neurology, University of Kentucky College of Medicine, Lexington, Kentucky 40536.

Administration of certain steroids during critical periods of development has been associated with anatomical changes in the cerebellum and hippocampus, where suppressive effects on postnatal granule cell genesis might disrupt critical periods of cell acquisition. Such changes have resulted in performance deficits of tasks which appear to be hippocampal dependent. The present study was designed to investigate changes in hippocampal anatomy which might contribute to such behavioral deficits.

Litters of Sprague-Dawley rats were culled to 8-10 animals and assigned to control or experimental treatment. The experimental animals received a single subcutaneous injection of dexamethasone (100 mg/kg) on day four of life. Control animals received an equivalent amount of saline. Animals were subsequently killed at 10, 20 and 60 days of age and the brains processed for histological analysis. One micron thick plastic sections were cut and stained with thionin and the density of pyramidal cells in the CA1 hippocampal subfield and granule cells of the dorsal leaf of the dentate gyrus was determined for each age group.

The control animals demonstrated a significant maturational change in neuronal density in the pyramidal cell layer of the hippocampal CA1 region. The pyramidal cells in control animals decreased in density from 10 to 20 days and by 20 days of age attained normal adult values. The dexamethasone-treated animals showed a similar decline in cell packing density which was more prolonged. At 10 and 20 days of age this group had significantly higher cell packing density than controls. By 60 days of age the pyramidal cell packing desnity was equivalent to controls.

The maturational pattern of the dentate granule cells was different from that of the pyramidal layer. No changes in cell packing density were seen in the development of the granule cell layer in control animals. In steroid-treated animals, a pronounced increase in cell packing density was seen at 10 days with a decline to control density by 20 days of age. This return to control values of cell density occurred in pyramidal and granule cell layers of experimental animals despite significantly lower total brain and hippocampal weights.

These data suggest that regulatory mechanisms of packing density of neurons are different in pyramidal and granule cell populations of the hippocampal formation. The granule cell population may exhibit more rapid adaptation to a postnatal insult, perhaps related to its capacity to regulate postnatal neurogenesis.
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249.6 ALPHA-MSH: BRAIN-PITUITARY INTERACTIONS IN THE DEVELOPMENT OF CENTRAL CONTROL OF PEPTIDE SECRETION. W. Lichtensteiger, M.D. Davis*and M. Schlumpf. Pharmacology Inst., Univ. of Zürich, CH-8006 Zürich, Switzerland.

The complex of tubero-hypophyseal dopamine (DA) neurons and pituitary intermediate lobe provides an opportunity to study developmental steps in central control of pituitary function. In Sprague Dawley rats, serum MSH levels (bioassay on Rana pipiens skin) exhibit marked changes during postnatal development, with a peak at postnatal days (PN) 5 and 6 (PN 1 = day of birth), low levels in the 2nd postnatal week and a rise thereafter. Data on ^{3}H-spiperone binding and in vitro release of MSH indicate that the intermediate lobe is responsive to DA at around birth (Davis et al., Neuroscience Abst. Vol. 7, p.290, 1981). We investigated possible relationships between variations in serum MSH and developmental processes in the central control of MSH.

The innervation of rat intermediate lobe by DA fibers develops slowly after birth. Fluorescent histochemical and biochemical analyses revealed that maximum density of DA innervation is reached at the end of the 2nd postnatal week when serum MSH is low. Neurointermediate lobe DA (COMT assay) rises from 1.07 $\pm$ 0.15 ng/mg protein at PN 3 to 31.7 $\pm$ 3.5 ng/mg at PN 15 and decreases to 7.01 $\pm$ 1.9 ng/mg at PN 90. The postnatal MSH peak appears to be linked with the onset of DA control: When pups were injected with flupenthixol (1 mg/kg i.p) at different times, serum MSH increased at PN 8, 15 and 22, but not at PN 5 (time of MSH peak) or PN 3 (before the peak). Moreover, microfluorimetric studies showed that the tubero-hypophyseal DA neurons responded to i.p injections of alpha-MSH at PN 8 but not at PN 4. This indicates that tonic inhibition of MSH secretion and central feedback loop are established at the end of the 1st postnatal week. Recent observations suggest that peripheral MSH in turn is important for the development of central control: Intravenous injections of anti-alpha-MSH on the two days of the postnatal MSH peak interfered with the development of the sensitivity of DA neurons to feedback effects of alpha-MSH.

249.7 HORMONAL REGULATION OF SYMPATHETIC DEVELOPMENT. R.W.Hamill and L.A.Guernsey*. Neurology Research Laboratories, Mon Com Hosp/ Univ of Roch Med Ctr., Roch, NY 14603

The effects of neonatal castration on neuronal ontogeny was examined in peripheral sympathetic ganglia in male Sprague-Dawley rats. Tyrosine hydroxylase (T-OH) activity, the rate limiting enzyme in catecholamine biosynthesis and a marker of noradrenergic maturation, was examined in the hypogastric (HG) and superior cervical ganglion (SCG).

Initial studies characterized the normal development of T-OH activity in HG ganglia. Enzyme activity increased to 70 fold during the course of postnatal ontogeny, reaching a plateau at the 60-70 days of age, the time of sexual maturity in these animals. Previous studies (Black et al '71) describe the normal ontogeny of SCG enzyme activity. Neonatal castration at 10-11 days of age prevented the normal ontogeny of HG T-OH activity: T-OH activity failed to develop normally and was 17% of sham-operated littermate controls when examined at 8 weeks of age, and less than 5% when studied 10 weeks after surgery. Total ganglion protein also failed to develop normally, but the magnitude of this effect was less than that observed for T-OH activity. Consequently, there was a specific deficit in the ontogeny of enzyme specific activity. In contrast to the effects in HG, there was no change in enzyme activity in the SCG.

Replacement therapy with testosterone decanoate completely reversed the developmental alteration in enzyme activity. Testosterone decanoate (TD)(20 mg/kg) was given on the day of surgery and every two weeks until 10 weeks after surgery. Vehicle treated sham-operated and castrated littermates served as control. Parenteral TD treatment reversed the developmental deficit. In order to examine whether a critical time existed during which HG neurons required hormonal exposure, animals were castrated as neonates but TD treatment was delayed until 8 weeks after surgery, 2 weeks before sacrifice. Hormone treatment restored T-OH activity to approximately 50% of levels in sham-operated littermates, whereas vehicle treated littermates remained at less than 5%.

These observations suggest that hormonal factors modulate noradrenergic ontogeny in peripheral sympathetic ganglia but appears restricted to ganglia whose targets include hormonally dependent sex organs. Additionally, enzyme deficits may be fully reversed if parenteral treatment is initiated early and partially reversed even if treatment is delayed for 2 months.

Black, IB, et al: Brain Res 34(1971) 229-240

249.8 ALTERATIONS OF BRAIN GLUCOSE METABOLISM IN CRETINISM. D.L. Dow-Edwards, A. Crane*, C. Kennedy, L. Sokoloff. Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda MD 20205.

The importance of thyroid hormone in brain development is well established. Eayrs (J. Endocrinol., 22:409-419, 1961) correlated the degree of neural impairment with the age of onset of thyroid deficiency in the rat. Rats thyroidectomized at birth show the greatest degree of aberrant reflexes, locomotion, and learning capabilities. Morphological analysis of cretin brains reveals a hypoplasia of the neuropil characterized by a decrease in arborization and synaptogenesis. There is a decreased cortical thickness, especially in layer 4, and a 45% reduction in capillary surface area. Most of the enzymes studied exhibit altered activity. In order to identify the distribution of the effects and to localize particularly vulnerable regions, the [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization was applied to cretinous rats.

Newborn rats were made cretinous by injecting 100 µCi ^{131}I ip and were housed with their mothers under the same conditions as their saline-injected littermates. At 5 months of age, animals were subjected to the 2-deoxyglucose method as previously described (Sokoloff, L., et al., J. Neurochem., 28:897-916, 1977). At this time plasma samples for $T_3 + T_4$ determination were taken to verify the thyroid status of the animal. Of the 23 brain regions and 10 animals analyzed thus far, all cretinous brains showed reduced glucose utilization as compared to controls. The metabolic rate in the cortical regions was reduced from 50-60%. Brain stem regions like the vestibular nuclei and the medial raphe were relatively less affected (33-35%). Of the sensory systems, the auditory system showed the greatest decrease in metabolic rate, with a 60% reduction in the inferior colliculus and auditory cortex. The inferior colliculus also showed an altered autoradiographic pattern of glucose utilization. In contrast, the lateral habenular nucleus was less affected in the cretinous state. These results demonstrate that hypothyroidism during development results in a generalized reduction in glucose utilization with the cortical structures and auditory system being particularly vulnerable.

249.9 GLUCOCORTICOID REGULATION OF SYNAPTIC DEVELOPMENT . Donald G. Puro. Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, MD 20205

The development of a functioning synapse is the result of a precisely timed sequence of steps. A central problem in neurobiology is to understand how the timing of events in synaptic maturation is regulated. I have used a cell culture system to explore the effect of glucocorticoid hormones on the developmental step in which a presynaptic neuron acquires the ability to transmit excitatory information across a synapse.

Cholinergic neurons dissociated from the perinatal rat retina form functional synapses in culture with rat striated muscle cells. Myotubes were used as postsynaptic targets since their membranes have areas with a high density of cholinergic receptors and because their response to acetylcholine has been well studied both in vivo and in culture. Also, their relatively large size permits prolonged intracellular monitoring of postsynaptic responses.

This retina-muscle cell culture system is well suited for the examination of specific steps in the development of a synapse. Early in the functional maturation of these retina-muscle synapses, there is a period in which release of acetylcholine occurs spontaneously, but cannot be evoked. This stage is followed by the emergence of transmitter release that is stimulus-evoked and dependent on extracellular calcium.

Here, I report that glucocorticoid hormones accelerated this developmental sequence. The half-maximally effective concentrations were 7nM for dexamethasone and 20nM for hydrocortisone. Experimental findings indicate that this induction is mediated by glucocorticoid receptors, acts at the transcriptional level, requires protein synthesis, and involves the depolarization-coupled release of transmitter.

This hormonal effect is not restricted to manipulation of culture conditions since the acceleration of the functional maturation of cholinergic neurons can occur in utero. If maternal glucocorticoid levels were elevated for a period during the 17th to 20th days of gestation by injecting dexamethasone or by producing stress (cold exposure), then cholinergic retinal neurons from the fetal retina formed synapses that were more functionally mature. Thus, the functional maturation of fetal synapses may be influenced by maternal stress.

The results indicate that glucocorticoid hormones can regulate the timing of the development step in which cholinergic neurons of the rat retina become capable of releasing acetylcholine at synapses in response to excitatory stimulation.

250.1 THE TOPOGRAPHIC ORGANIZATION OF THE RUBROSPINAL TRACT IN THE RHESUS MONKEY. T.C. Truscott and N.L. Strominger. Department of Anatomy, Albany Medical College, Albany, NY 12208.

Stereotactic injections of tritiated leucine were placed in the red nucleus of 13 monkeys using a posterior fossa craniotomy. Animals were sacrificed 4-14 days following surgery by transcardial perfusion with 0.9% saline followed by 10% neutral formalin. Brains were blocked in the transverse plane of the brainstem. Most cases were post-fixed in 30% sucrose-10% formalin and sectioned at 20μm on a freezing microtome. A few cases were embedded in paraffin. Sections were exposed with Kodak NTB-2 Nuclear Track Emulsion for 4-22 weeks, developed with Kodak D-19 and stained with cresyl violet.

Injections involving the caudal coarse-celled portion of the red nucleus (RNc) produced label which coursed in the rubrobulbo-spinal tract (RST). Injections restricted to the rostral part of the red nucleus, as well as those behind the RNc, did not produce label in the RST. Labeled fibers of coarse caliber decussated at the level of the red nucleus and passed caudally as a compact bundle in the ventral and then ventrolateral part of the brainstem. Some fascicles passed dorsally from the RST into and especially along the lateral margin of the facial nucleus. This label was particularly evident in slides exposed for 22 weeks. Copious label invariably passed into the dorsal parvocellular division of the lateral reticular nucleus, but avoided the magnocellular division. Some label continued through the nucleus in a dorsomedial direction into the medullary reticular formation.

Cases with injection sites localized in the ventral part of the RNc contained label which entered the lateral quadrant of the spinal cord and descended its entire length. Fine caliber label was located primarily over the intermediate grey and dorsal horn. In contrast, injections involving the dorsal part of the RNc produced label with a similar distribution in the spinal cord which could be followed only through cervical levels.

In some cases label was followed dorsally from the rubrospinal tract along the lateral border of the principal sensory trigeminal nucleus and the superior cerebellar peduncle. This label passed into the cerebellum in association with the uncinate fasciculus and was distributed to the interposed nuclei. Label was not present in the principal sensory trigeminal nucleus or the dorsal division of the motor trigeminal nucleus. Some label came into geographic relationship with the ventral division of the motor trigeminal nucleus.

Supported in part by NIH Grant 12208.

250.2 A DOUBLE LABELLING STUDY DEMONSTRATING THAT MOST CELLS IN THE NUCLEUS RETICULARIS GIGANTOCELLULARIS AND ADJACENT RAPHE PROJECT TO EITHER THE ANTERIOR LOBE OF THE CEREBELLUM OR THE SPINAL CORD IN THE RAT. R.P. Waltzer* and G.F. Martin (SPON: K. Michal). Dept. of Anat., Coll. of Med., Ohio State Univ., Columbus, OH 43210.

HRP data show that the nuclei reticularis gigantocellularis and obscurus raphae project to the anterior lobe of the cerebellum as well as to the spinal cord in the rat. It appeared possible that cells in these regions might provide collateral innervation to both the cerebellum and the spinal cord. In order to address this issue we employed the retrograde transport of fluorescent dyes in double labelling experiments. The forelimb region of the spinal cord (the cervical enlargement) received injections of True Blue (TB) followed 7 days later by injections of Nuclear Yellow (NY) into the presumed forelimb region of the anterior lobe. In other experiments the hindlimb region of the spinal cord (the lumbar enlargement) received injections of TB followed 5-8 days later by injections of NY centered in the presumed hindlimb region of the anterior lobe. The animals were sacrificed 24 hours or less after the second injection. The brains were removed, frozen on dry ice and sectioned on a cryostat. The sections were examined with a fluorescence microscope using an excitation wavelength of 360 nm. Cells which projected to the spinal cord contained TB in their cytoplasm, while those innervating the cerebellum contained NY in their nuclei. Those which projected to both regions contained TB in their cytoplasm and NY in their nuclei. Neurons projecting to the spinal cord were found throughout the rostral-caudal extent of the nucleus reticularis gigantocellularis, whereas those projecting to the cerebellum within the same nucleus were found only at intermediate rostral-caudal levels. TB labelled cells were interspersed with NY labelled cells within the nuclei reticularis gigantocellularis and obscurus raphae. Less than 2% of all labelled cells contained both markers. Similar results were obtained regardless of the injection combinations. Based on classical studies, we thought it likely that most of the reticular and raphe cells in question would provide collateral innervation to the anterior lobe of the cerebellum and to the spinal cord, but this was not the case. Further studies of collateral projections from reticular nuclei may be appropriate. (Supported by BNS-80-08675 and NS-08798.)

250.3 DETAILED MORPHOLOGY OF CAT PHRENIC MOTONEURONS. W.E. Cameron, D.B. Averill* and A.J. Berger. Dept. Physiol. and Biophysics, Univ. Washington, Seattle, WA 98195.

The anatomical techniques that have been previously utilized to study phrenic motoneurons (PMs) were inadequate to describe the detailed ramifications of the dendritic tree or the existence of axon collaterals. We selected the technique of intracellular injection of horseradish peroxidase (HRP) to investigate the morphology of cat PMs. Cells were impaled with bevelled glass microelectrodes containing 4-10% solution of HRP in KCl-tris buffer. After achieving stable intracellular recording, the membrane potential was recorded during several respiratory cycles and during sub- and suprathreshold stimulation of the phrenic nerve. Then, HRP was injected into the cell using pulses of depolarizing current (10-20 nAmps). The cervical cord was fixed, frozen, sectioned and reacted with diaminobenzidine and hydrogen peroxide. Neuronal reconstructions were made using a drawing tube attachment to a light microscope.

When transverse sections were examined, the PMs were found to lie between the ventrolateral and ventromedial nuclei of Rexed's lamina IX. The dendrites coursed dorsomedially (2-5 crossing midline), dorsolaterally to laminae VII and VIII and into the lateral funiculus and ventrolaterally into the ventral funiculus. There was a paucity of dendrites immediately dorsal to the cell body. A large proportion of the dendritic tree was directed in a rostrocaudal axis extending up to 1500μm from the cell body. In sagittal and horizontal section the rostrocaudal dendrites exhibited a tight clustering within the phrenic motor column. Single-lobed dendritic spines were found along the dendrites projecting to each of the fields mentioned above. In several instances, multi-lobed spines were also observed.

When the HRP-filled motor axons were examined with a 100X oil immersion objective, there was no evidence of axon collaterals in either ventral grey or white matter. This finding was consistent with the electrophysiology. The subthreshold stimulation of the phrenic nerve did not reveal a hyperpolarizing wave in the stimulus-triggered average of the intracellularly recorded synaptic noise at a latency appropriate for recurrent inhibition.

This work was supported by NIH grant NS 14857 and a fellowship from the Muscular Dystrophy Association.

250.4 SCIATIC NERVE CONNECTIONS TO THE SPINAL CORD DETERMINED BY THE TRANSPORT OF HORSERADISH PEROXIDASE. S. L. Scharoun, F. C. Barone, P. A. McGrattan,* B. B. Falk,* S. M. Jones* and M. J. Wayner. Brain Research Lab., Syracuse Univ., 601 University Ave., Syracuse, NY 13210.

Crystals of pure horseradish peroxidase (HRP, Sigma Type VI) were encased in a parafilm envelope and applied to the transected central ends of the left and right sciatic nerves of adult male hooded rats. After a 48 hr survival time, animals were perfused intracardially with a phosphate buffer plus sucrose followed by a glutaraldehyde and paraformaldehyde fixative. Spinal cord segments and corresponding dorsal root ganglia were removed and cut into 50 μm sections. All tissue was processed with TMB and counterstained with neutral red for the blue reaction according to Mesulam. Sequential sections were examined under a microscope. Labeled neurons and nerve terminals were identified using bright and dark field condensers and polarized light. Retrogradely labeled bipolar neurons were identified in the dorsal root ganglia of the thoracic spinal cord. Anterograde terminal labeling was also identified in thoracic segments. Terminals were observed as a dense band in laminae I which continued to be concentrated medially from laminae II through IV. Lower portions of the thoracic dorsal horn contained a more diffuse distribution of labeled terminals. The lumbar segments of the spinal cord contained motor neurons located ipsilaterally in the ventral horn in laminae IX and in the ventrolateral portions of laminae VII. Also, the dorsal root ganglia of many lumbar segments contained labeled bipolar neurons. Terminal labeling in the lumbar dorsal horn was identified as a very think band in laminae I and distributed throughout laminae II-IV. These results illustrate the distribution of sciatic nerve connections in the lower spinal cord.

(Supported by NINCDS USPHS grant No. 13543.)

250.5 ULTRASTRUCTURAL ORGANIZATION OF THE HYPOGLOSSAL NUCLEUS OF THE RAT. T.B. Boone‡ J.G. Linner‡ and L.D. Aldes* (Sponsor: R.C. Wiggins.) Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77025.

Hypoglossal motoneurons must integrate diverse synaptic inputs to regulate the action of the tongue. An ultrastructural study of the hypoglossal nucleus is essential, therefore, in understanding the functional basis of tongue control.

Female, Sprague-Dawley rats were perfused-fixed with 0.9% sodium chloride followed by a 0.1 M cacodylate buffered (pH 7.4) glutaraldehyde (1.0%) and paraformaldehyde (1.0%) solution. Vibratome sections (100 um) through the hypoglossal nucleus were taken in either the coronal or sagittal plane, osmicated, dehydrated and embedded in Spurr. Thin sections were cut, stained with uranyl acetate and lead citrate and examined in a JEOL-100B electron microscope.

Low magnification photomicrographs through the hypoglossal nucleus revealed a highly vascularized neuropil consisting of motoneuron cell bodies, numerous perineuronal astrocytes and oligodendrocytes and a wealth of cell processes oriented predominantly in a rostrocaudal direction. Axons ranging from heavily myelinated to non-myelinated were observed, and dendrites of varying sizes were seen in contact with a diversity of synaptic endings.

The ultrastructural morphology of hypoglossal motoneurons was characterized by: 1) an abundance of cytoplasm surrounding a large, centrally located nucleus with a prominent nucleolus; 2) aggregations of granular endoplasmic reticulum arranged in lamellae and distributed throughout the soma and proximal dendrites; 3) an array of other cellular organelles including lysosomes, golgi complexes, dense bodies and glycogen granules found throughout the cytoplasm; and, 4) numerous mitochondria, microtubules and neurofilaments in both soma and cell processes.

A diverse group of synaptic endings was seen to contact dendrites and motoneuron cell bodies. Three types of synaptic terminals were observed. The first type was characterized by clear, spherical vesicles of uniform size. A postsynaptic density was typically associated with this type of ending. Although most synapses (axo-dendritic or axo-somatic) were of this type, dense core granules or subsynaptic dense bodies also were observed. Pleomorphic vesicles characterized the second type of synaptic ending, while flattened vesicles constituted the third type of ending. Both of these endings were associated with a symmetrical synapse. No axo-axonal synapses were observed.

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250.6 ORGANIZATION OF FROG MESENCEPHALIC TEGMENTAL NUCLEI. L.J. Prior and W.L.R. Cruce. Neurobiology Program, NEOUCOM, Rootstown, OH, 44272.

The frog mesencephalic tegmentum has been divided into cellular groups largely on cytoarchitectonic grounds. Potter (1964) described four tegmental nuclei - Posterodorsal (Pd), Posteroventral (Pv), Anterodorsal (Ad), and Anteroventral (AV). We have become particularly interested in describing the connections of two of these nuclei: Pv, which contains cells of the lateral portion of the superior reticular formation, and Av, which contains two cellular groups: a ventromedial group which represents the nucleus of the fasciculus longitudinalis medialis, and a larger celled dorsolateral group which probably represents the anuran homologue of the nucleus ruber. Both *Rana Catesbeiana* and *Rana Pipiens* were used in two series of experiments. In the first series horseradish peroxidase (HRP) was applied to various spinal cord levels either as micropipette injections of 1 ul of 40 HRP in 1 lysolethicin or as small blocks of gelfoam soaked in 40 HRP. The second series consisted of pressure injections of 10-20 nl of 20-30 HRP into various tegmental regions. Survival times ranged from 2-14 days. Tissue was processed with the tetramethyl benzidine method for localizing HRP. Following spinal cord injections, cells were found (1) contralaterally in Av at the level of the oculomotor nucleus, (2) ipsilaterally in ventromedial Av rostral to the level of the oculomotor nucleus, and (3) ipsilaterally in the central portion of Pv. Tegmental injections at the level of the oculomotor nucleus showed cellular label in a eedially located cerebellar nucleus, and fiber label in the dorsolateral and ventromedial funiculi of the spinal cord. We find that Pv contains cells of the superior reticular formation in its central part. Av contains two distinct cell groups each of which projects to spinal cord - one in the ventromedial funiculus, the other in dorsolateral white matter. We suggest that the rostrally located ventromedial group represents the nucleus of the fasciculus longitudinalis medialis, while the more caudally located dorsolateral group represents the anuran homologue of the nucleus ruber. (Supported in part by a Sigma Xi Grant-in-Aid of Research to L.J.P. and B.R.S. Grant no. 2S07 RR05 806 to W.L.R.C.

250.7 IDENTIFICATION OF MOTONEURONS INNERVATING THE LEVATOR VELI PALATINI MUSCLE IN THE CAT. H. van Loveren*, J.T. Keller, and M.C. Saunders*. Dept. of Anatomy, University of Cincinnati, Dept. of Neurosurgery, The Christ Hospital and The Christ Hospital Institute of Medical Research, Cincinnati, OH 45219.

Recent investigations of the nucleus ambiguus (NA) have attempted to identify motoneurons associated with the branchiomeric muscles of the larynx and pharynx. Relatively little attention has been directed to the levator veli palatini muscle which is critical in certain respiratory functions. Although consensus today is that cranial nerve X innervates this muscle, some investigators have suggested that the levator veli palatini muscle is innervated by either cranial nerve VII or IX. The purpose of this study was to identify motoneurons giving rise to fibers innervating the levator veli palatini muscle.

Using a Hamilton syringe, 10-20ul of 30% horseradish peroxidase (HRP, Sigma Type VI) was injected indirectly into the levator veli palatini muscle in 11 cats (soft palate injection), and directly into the muscle in 4 cats. Twenty-four to 48 hours after injection the animals were sacrificed and fixed by perfusion. Tissue blocks cut at 40um were processed according to Mesulam's technique for tetramethylbenzidine (TMB).

HRP-TMB reaction product was identified in cells located in the NA both ipsilateral and contralateral to the side of injection. Labeled cells were located as far caudal as the rostral pole of the hypoglossal nucleus and extended rostrally to the level of the rostral pole of the inferior olive. The caudal-most portion of the NA contained no HRP labeled cells. A few labeled cells were located in the retrofacial nucleus, suggested by Taber to be the ventral portion of the rostral continuation of the NA.

HRP labeled cells in the region of the rostral hypoglossal nucleus and caudal nucleus prepositus were few in number and loosely arranged. At more rostral levels, the NA appears as a discrete, densely arranged cluster of cells. In this area HRP labeled cells are more numerous and uniformly distributed within the nucleus. In the NA contralateral to the side of injection, labeled cells were in a similar location and arrangement, but fewer in number and did not extend as far rostral as those on the ipsilateral side.

This is the first HRP demonstration of motoneurons associated with the levator veli palatini muscle. Location of HRP labeled cells within the NA excludes innervation by cranial nerve VII. The rostral compact portion of the NA has been associated with muscles of the palate in the rabbit, whereas glossopharyngeal motoneurons have been associated with the loosely arranged medial portion of the NA. Therefore, location of HRP labeled cells within the compact lateral portion of the NA supports the view that the levator veli palatini muscle is innervated by cranial nerve X. Innervation of this muscle appears to be bilateral.

250.8 COMMAND PATHWAYS AND SENSORY GATING OF SWIMMING MOTOR ACTIVITY IN THE LAMPREY. A.D. McClellan and S. Grillner, Dept. of Physiol. III Karolinska Institutet, Lidingövägen 1, 114 33 Stockholm, Sweden

Two in vitro preparations of the lamprey (Ichthyomyzon unicuspis) have been developed for examining the neural organization of escape swimming (McClellan and Grillner, Neurosci. Abst. 1981). (1) A brainstem preparation consisting of the head and exposed brainstem attached to 20-30 segments of the isolated spinal cord/notochord. (2) A tail preparation consisting of 2-3 cm of the tail fin attached to 30-40 segments of the isolated spinal cord/notochord. Mechanical pressure (i.e. pinching) applied to the snout or tail fin in these curarized preparations elicits a "fictive" escape swimming pattern in pairs of rostral and caudal ventral roots.

Both preparations can be partitioned into chambers which are isolated by barriers sealed with Vaseline. In this way, special ionic solutions or synaptic transmitter blockers can be placed independently in the various chambers. For example, with a low Ca^{++} solution in the middle region of the spinal cord, so as to locally block chemical synaptic transmission, snout or tail fin stimulation still initiates swimming motor activity in the part of the spinal cord beyond the region of synaptic blockage. Thus, the descending and ascending activation pathways for swimming pass directly from their origin to the spinal pattern generator networks, and chains of serially connected short neurons are not needed.

With both of the above preparations, the part of the spinal cord/notochord furthest from the point of mechanical stimulation can be bent laterally to determine the effects of proprioception on elicited swimming motor activity. In all cases, tonically bending the mobile part of the preparation results in ipsilateral gating of the first bursts of swimming activity in ventral roots. Thus, stimulation of an animal whose body is already partially bent will result in further bending in the same direction (as part of an initial withdrawal response), followed by escape swimming. Sensory inputs are therefore capable of selectively gating the swimming responses initiated by descending and ascending activation pathways.

Finally, with both preparations it is possible to investigate the location of activation pathways in the spinal cord. For example, the ascending pathways do not seem to be confined to a single region in the spinal cord, as lesions of the medial (i.e. dorsal columns), intermediate, or lateral regions of the cord do not abolish swimming activity initiated by tail fin stimulation.

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250.9 *THE EFFECT OF* SUBTOTAL SPINAL CORD LESIONS ON LOCOMOTION IN THE STINGRAY, DASYATIS SABINA. B.J. Williams, C.A. Livingston and R.B. Leonard. Marine Biomedical Institute and Dept. of Physiology and Biophysics, Univ. of Texas Medical Branch, Galveston, Texas 77550.

Stingrays which have undergone a complete high spinal transection do not locomote, even in response to strong exteroceptive stimulation. Previous studies have shown that electrical stimulation of the dorso- and ventrolateral funiculus in a high spinal stingray preparation can produce a replica of the locomotor output seen in the intact animal. We now report the effects of subtotal spinal cord lesions on locomotor activity in freely behaving as well as restrained stingrays.

For each animal, electromyographic records of locomotor activity were made both before and after the spinal cord lesion. Each animal was anesthetized and a series of EMG electrodes were inserted in the elevator and depressor muscles of one or both pectoral fins. The animal was allowed to recover from anesthesia and the EMG activity was recorded during spontaneous restrained swimming. The animal was reanesthetized and a laminectomy was performed over spinal segments 3-8. Lesions of the spinal cord were made either electrolytically or by aspiration. Typically the animals were allowed to recover at least one day following surgery before EMG electrodes were reinserted and the locomotor behavior (if any) was recorded. Analyses of the intersegmental delay between dorsal and caudal recording sites and the cycle period were compared for both intact and post-operative locomotor behavior. The extent of the lesions were determined by examination of Kluver-Barrera stained paraffin sections through the site of the lesion.

Stingrays which have undergone a hemisection of either the right or left half of the spinal cord can swim spontaneously using both pectoral fins. Most of the values obtained in the relation between intersegmental lag and cycle period in the post-operative swim overlap those obtained in the intact animal. This relationship also remains the same in animals in which the medial one-third of the cord is interrupted, including the ventral funiculus. Similarly, no change occurs in the organization of the spontaneous swim of animals which have undergone a hemisection on one side plus a lesion of either the dorsal or ventral portion of the lateral funiculus. Animals in which large portions of both lateral funiculi are damaged either require strong exteroceptive stimulation in order to initiate swimming or do not swim at all.

Hence, in agreement with the previous stingray spinal cord stimulation studies, the descending pathway necessary for the initiation of locomotion lies in the lateral funiculus of the spinal cord. Whether multiple pathways or one large and relatively diffuse pathway in the lateral funiculus subserves the initiation of locomotion remains to be determined.

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250.10 **MORPHOLOGY AND INNERVATION OF M. RETRAHENS CAPITIS COLLIQUE IN A TURTLE, PSEUDEMYS SCRIPTA.** M. Yeow* and E. H. Peterson (SPON: C. Straznicky). School of Anatomy, University of New South Wales, Sydney, Australia.

M. retrahens capitis collique (RCCQ) is a complex muscle which is believed to act as the chief retractor of the head and neck in Pseudemys. It is composed of two bellies: a long belly attached caudally to the centra of the sixth through ninth dorsal vertebrae and cranially to the pterygoid and basisphenoid; and a short belly which arises caudally along the centra of the fifth through ninth dorsals and is attached by three slips to the transverse processes of C4-C6.

As part of a larger study of the cervical musculature in Pseudemys we characterized movements of the vertebral column during head retraction and the motor neurons supplying this muscle. To determine the correct alignment of cervical vertebrae during normal protraction and retraction, radiographs were made of an awake, unanaesthetized turtle. Our data indicate that during head retraction the sixth, fifth and fourth cervicals which give attachment to the short belly are brought successively caudal and dorsal, forming a C-shaped curve with the concavity facing anteriorly. In full retraction C4 is the caudalmost vertebra of the cervical complex. Examination of these vertebrae indicates that they are united by ellipsoid (4/5) or bicondylar joints (5/6, 6/7) which tend to restrict movement to a hinge like rotation in the sagittal plane. Thus RCCQ appears to effect retraction by a caudal pull on the transverse processes which swings each of these 3 vertebrae around a transverse axis through their caudal intercentra articulations.

In seven animals, HRP was applied to one or both bellies of the muscle. The resulting pattern of label differed from that of the other superficial cervical muscles in two ways. First, the muscle is supplied by virtually the entire cervical spinal cord (C2-C8) whereas the other neck muscles are typically innervated by one to three segments. Second, the muscle is supplied by two motor nuclei: the lateral subcolumn of the ventral nucleus and the medial (commissural) nucleus. Medial nucleus cells have primary dendrites which extend across the midline in the ventral commissure. They supply Spinalis Cervicis and Longus Colli, in addition to RCCQ, and may provide a non-musculotopic system for producing bilaterally synchronous muscle contraction (Peterson & Yeow, '81). This pattern of innervation via multiple nuclei contrasts with that seen in the other superficial neck muscles which tend to be supplied by a single motor nucleus. We now wish to determine whether the different motor nuclei supply preferentially different histochemical fiber types within the same muscle, as appears to be the case in certain neck muscles of rats (Brichta & Peterson, '82).

250.11 ORGANIZATION OF MOTOR POOLS SUPPLYING CERVICAL MUSCULATURE IN THE RAT. A. Brichta* and E.H. Peterson (SPON: J. Stone). School of Anatomy, University of New South Wales, Sydney AUSTRALIA

The organization of motor pools supplying seven cervical muscles in rats was examined using Nissl stained preparations cut in transverse and horizontal planes, and retrograde transport of horseradish peroxidase (HRP) applied to individual neck muscles or to single muscle nerves.

The superficial ventral muscles (Sternomastoid, Cleidomastoid, Cleidotrapezius) are supplied by three motor nuclei. In the medullary transition zone, between the sensory decussation and C1 ventral root, all three muscles are supplied by motorneurons in the commisural and centrodorsal nuclei of Rexed. Just caudal to the C1 ventral root, the location of the common motor pool shifts laterally to the region of the (spinal) accessory nucleus. Here motorneurons of Sternomastoid and Cleidomastoid occupy the same subcolumn; Cleidotrapezius cells are located somewhat more dorsolaterally, in the same subcolumn as the dorsal neck muscle Acromiotrapezius, but at more rostral levels. In these experiments, labelled cells in the centrodorsal nucleus are significantly larger than those in the accessory nucleus: e.g. 29±4um vs. 22.8±3um for Cleidotrapezius; 26.5±4.9um vs. 23.6±3.6um for Sternomastoid. Application of HRP to the white segment of Sternomastoid which contains more than 90% fast/glycolytic fibers labels only cells of the centrodorsal nucleus; in contrast, the red segment of Sternomastoid contains primarily fast/oxidative with a few (less than 5%) slow twitch fibers, and is innervated by a population of smaller neurons in the accessory nucleus. Filled axons from centrodorsal and accessory cells join together to form the spinal accessory nerve. Thus the centrodorsal nucleus appears to be a medial subdivision of the spinal accessory complex which is specialized for innervating the fast/glycolytic fibers of these muscles. The location of this nucleus in the terminal field of the tectospinal tract (Waldron & Gwyn, '69) suggests that the white segments of the superficial ventral muscles are under synaptic drive from the superior colliculus and that they may be preferentially involved in rapid head orienting movements mediated by the colliculus. The fourth muscle supplied by the spinal accessory complex, Acromiotrapezius, is innervated exclusively by the accessory nucleus.

Motor pools of three deep dorsal neck muscles (Splenius, Biventer Cervicis, Complexus) are located in the ventromedial nucleus of Rexed. These motorneurons are located in two subcolumns: Splenius neurons are relatively more dorsal, whereas Biventer Cervicis and Complexus neurons occupy the same, more ventral, cell column. We were unable to confirm the report of Richmond et al. ('78) that the deep dorsal muscles are also supplied by cells of the commisural, accessory and centrodorsal nuclei.

250.12 HISTOCHEMICAL PROFILES OF CERVICAL MUSCLES IN THE RAT. R. Callister*and E.H. Peterson (SPON: M. Girgis). School of Anatomy, University of New South Wales, Sydney AUSTRALIA

Two groups of cervical muscles in rats, the spinal accessory complex (Sternomastoid, Cleidomastoid, Cleidotrapezius, Acromiotrapezius) and the deep dorsal muscles (Splenius, Biventer Cervicis, Complexus), were analyzed to determine their histochemical composition and the topography of each fiber type within the muscles, using serial sections stained for alkaline stable myosin ATPase and succinic dehydrogenase. Our results suggest that there are two patterns of internal organization in the cervical muscles.

Muscles on the ventral aspect of the neck (Sternomastoid, Cleidomastoid, Cleidotrapezius) are 90-98% fast twitch. They differ primarily in the proportion of oxidative fibers from Cleidotrapezius which has less than 25% slow plus fast/oxidative fibers to Cleidomastoid which is primarily (60%) oxidative. In all three muscles there is a sharp segregation of fibers into white (more than 90% fast/glycolytic) and red (oxidative) segments. The oxidative compartments of the three muscles are opposed and when viewed in situ they appear to form an oxidative core surrounded by an annulus of fast/glycolytic fibers. The red and white segments appear to be innervated by different motor nuclei and to be under different patterns of supraspinal drive (Brichta & Peterson, '82).

Muscles forming the dorsal neck mass (Acromiotrapezius, Splenius, Biventer Cervicis, Complexus) present a different histochemical profile. They are characterized by a relatively high proportion of slow twitch fibers: 10-12% in Acromiotrapezius and Splenius; 12-15% in Complexus and 25-29% in Biventer Cervicis. In contrast, slow twitch fibers in the ventral group average approximately 5%. Furthermore there is no sharp compartmentalization of fiber types; there is a slight tendency for slow and fast/glycolytic fibers to have a complimentary distribution, but fast/oxidative fibers are distributed evenly throughout the muscles. Thus the dorsal neck muscles have a relatively high and spatially uniform oxidative capacity.

Our data suggest that the dorsal and ventral muscle groups can also be distinguished on the basis of muscle spindle distribution. In the ventral group, muscle spindles are restricted to the oxidative compartments; there are no muscle spindles in the white segments. In contrast, spindles are distributed more homogenously throughout the deep dorsal muscles although there is a slight tendency for them to cluster toward the medial aspect (Splenius) or the core (Complexus, Biventer Cervicis) of the muscle. Thus muscle spindle distribution in the cervical complex mirrors that of the slow twitch fibers.

250.13 THE MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED CORTICOSPINAL AXONS IN THE CAT, A.G. BROWN,* R.E.W. Fyffe,* D.J. Maxwell,* and H.J. Ralston III. Dept. Veterinary Physiology, Univ. of Edinburgh and Dept. Anat., Univ. Calif., San Francisco, CA 94143.

Intraaxonal staining with horseradish peroxidase (HRP) was used to demonstrate the morphology of cat corticospinal axons. Glass micropipettes filled with 5% HRP were used to impale single axons in the dorsolateral column white matter of the lumbar enlargement. Axons which followed single shocks and 100 Hz stimulus trains applied to the contralateral pericruciate cortex with a surface electrode were characterized as corticospinal axons. The fibres were labelled by iontophoreses of the HRP, and 12 different collaterals which arborized in the spinal gray matter were subsequently examined by light and electron microscopy.

Labelled stem axons in the dorsolateral column white matter were found to give off several collateral branches to the spinal gray, at intervals of 300-600 μm. The collaterals took a ventromedial course to enter the lateral aspect of lamina V or VI. Each collateral arborized extensively in the gray, often exhibiting boutons en passage or termineaux in more than one lamina. The major arbors were found in laminae V, VI, VII and IX. Electron microscopy of the terminations of corticospinal neurones shows that they form axo-dendritic and axo-somatic synapses; six boutons were observed on the prominal dendrites and soma of one lamina VI cell. Usually only one synaptic junction is formed per bouton but occasionally two dendrites were seen to be postsynaptic to one bouton.

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251.1 ATP-DEPENDENT GLUTAMATE UPTAKE INTO PROTEIN I-ASSOCIATED SYNAPTIC VESICLES. S. Naito* and T. Ueda. Mental Health Research Institute Depts. of Pharmacology and Psychiatry, University of Michigan, Ann Arbor, MI 48109.

Protein I is a neuron-specific, major endogenous substrate for both cyclic AMP-dependent and calcium/calmodulin-dependent protein kinases present in the synaptic region, and is associated with the surface of synaptic vesicles in highest concentrations. There is evidence that the state of phosphorylation of Protein I in presynaptic terminals is increased by the putative neurotransmitters serotonin and dopamine, through an increased formation of cyclic AMP, and by depolarization and physiologically relevant electrical impulses, through an increased influx of calcium. These lines of evidence have suggested that Protein I may be involved in regulation of neurotransmitter release. However, the types of neurotransmitter present in Protein I-associated synaptic vesicles have not been identified biochemically.

We have recently isolated anti-Protein I IgG by affinity chromatography and showed that these antibodies inhibit specifically the phosphorylation of Protein I (J. Biol. Chem. 256, 10657-63, 1981). In an effort to characterize Protein I-associated synaptic vesicles with respect to the types of neurotransmitters, we have now developed a procedure, using the affinity-purified anti-Protein I IgG, which allows the isolation of those synaptic vesicles which contain Protein I. We present evidence that the isolated Protein I-associated synaptic vesicles from bovine cortex are able to accumulate specifically L-glutamate in an ATP-dependent, temperature-dependent but Na-independent manner. The ATP-dependent glutamate uptake requires ATP hydrolysis, since there was little accumulation of glutamate in the absence of Mg^{2+} or when ATP was replaced by an unhydrolyzable β,γ-methylene ATP analog. The glutamate uptake appears to be driven by a transmembrane pH gradient and/or membrane potential generated by Mg-ATPase in a mechanism similar to that of the catecholamine uptake into synaptic vesicles and chromaffin cell granules. Thus, agents known to dissipate a transmembrane proton gradient, such as FCCP, methylamine, and nigericin in the presence of potassium ions, caused a marked inhibition of the ATP-dependent uptake of glutamate into Protein I-associated synaptic vesicles. Moreover, the L-glutamate uptake was not significantly inhibited by L- or D-aspartate, L-glutamine, or GABA; and all other putative neurotransmitters tested failed to show an ATP-dependent uptake. The Km and Vmax of the L-glutamate uptake system were determined to be 1.56 mM and 13 nmol/min/mg, respectively. These observations suggest that Protein I-containing synaptic vesicles are associated with glutamate as a major neurotransmitter in the central nervous system. (Supported by USPHS Grant NS15113, from NINCDS.)

251.2 SPECIFIC BINDING SITE FOR [^{3}H]-GLUTAMIC ACID ON N18-RE-105 NEUROBLASTOMA AND ITS REGULATION. A.T. Malouf, R.L. Schnaar, and J.T. Coyle. Div. of Child Psychiatry and Depts. of Psychiatry, Neuroscience and Pharmacology, Johns Hopkins School of Medicine, Baltimore, MD. 21205.

The specific binding of [^{3}H]-glutamate (GLU) was characterized in washed membranes isolated from N18-RE-105, a neuroblastoma-retinal hybrid cell line. Cells were cultured in DMEM supplemented with 5% fetal calf serum and containing hypoxanthine, aminopterin and thymidine (HAT). Binding of [^{3}H]-GLU was performed in 50 mM Tris-HCL at 37° for 20 min, following which the membranes were isolated by centrifugation.

[^{3}H]-GLU bound in a saturable and reversible fashion with a K_D of 600 nM and a B_{max} of 12 pmole/mg prot. Pharmacologic characterization of the site indicates that it closely resembles the Na^+- independent binding site for GLU found on brain membranes and thought to be an excitatory amino acid receptor. Thus, while kainate, N-methyl-D,L-aspartic acid and non-amino acid ligands did not displace [^{3}H]-GLU, quisqualate and ibotenate were potent inhibitors of specific binding. Furthermore, this binding site has regulatory properties which resemble those described in the hippocampus (Baudry and Lynch, Nature 282:748, 1979). As in the hippocampus, preincubation of the membranes in the presence of Ca^{++} for 10 min caused a dose dependent increase in specific binding that was the result of an increased number of binding sites with no change in their affinity. Maximal stimulation required Ca^{++} concentrations in excess of 1 mM and resulted in greater than 100% stimulation of binding. In contrast, preincubation with monovalent cations resulted in a decrease in the number of sites. La^{+++} was the only other cation which enhanced the specific binding of [^{3}H]-GLU.

N18-RE-105 cells grown in the presence of 10mM GLU for 48 hours exhibited significant reduction in cell number as measured by lactate dehydrogenase activity or protein content relative to cells grown in control medium. Membranes isolated from the GLU-exposed cells exhibited doubling in the number of binding sites for [^{3}H]-GLU with no alteration in K_D; binding site density was further increased by preincubation of the membranes with Ca^{++}. This regulation of the binding site after exposure to GLU, and the further increase seen after preincubation with Ca^{++}, is reminiscent of the effect of post tetanic stimulation in the hippocampus. In summary, N18-RE-105 cells possess a pharmacologically relevant binding site for [^{3}H]-GLU, which exhibits regulatory properties resembling those previously described in hippocampal membranes. (This research was supported by USPHS Grants NS-13584 and MH-00125).

251.3 KAINIC ACID SELECTIVELY INDUCES THE RELEASE OF ENDOGENOUS EXCITATORY AMINO ACIDS FROM BRAIN SLICES IN VITRO. J. W. Ferkany, Ph.D. and J.T. Coyle, M.D., Div. of Child Psychiatry and Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Whereas the neuroexcitatory actions of kainic acid (KA) are direct, its neurotoxic effects require the integrity of excitatory, presumably glutamatergic, afferents in the striatum. Receptors for KA, which are distinct from those mediating the action of glutamate (GLU), are located on both pre- and post-synaptic neuronal elements. Accordingly, the current studies were undertaken to investigate the influence of KA on the release of excitatory amino acids from brain slices incubated in vitro in an attempt to elucidate further the neurotoxic mechanism of KA.

Various brain regions from male albino mice or Sprague-Dawley rats were sectioned at 225 µm immediately after sacrifice and the tissue was preincubated (60 min, 37°C) in oxygenated Krebs-bicarbonate buffer. Portions of the tissue were then transferred to fresh buffer or a superfusion apparatus and exposed to the drugs of interest. Endogenous amino acids released into the buffer were measured by a precolumn derivatization HPLC method. Both KCl and KA (EC_{50}=1mM) caused a dose dependent release (200-1000%) of aspartate (ASP) and GLU but not glutamine from striatal, hippocampal and cerebellar slices. Removal of Ca^{++} from the medium inhibited (>70%) both the KCl and KA-induced release of ASP and GLU whereas tetrodotoxin (5µM) selectively attenuated the KCl but not KA-stimulated efflux of these amino acids.

To better localize the site of action of KA, cerebella from 10 day old rats, which lack a mature parallel fiber system, were employed. KA increased the efflux of several amino acids including ASP but did not stimulate the efflux of GLU. Similarly, in slices prepared from cerebella of adult granulo-prival mice, KA caused a release of ASP comparable to that observed from intact cerebella whereas the release of GLU was reduced by 75%. On the basis of these studies, the primary source in cerebellum of KA-stimulated GLU efflux appears to be the granule cell-parallel fiber system. The KA-induced stimulation of ASP and GLU release was not mimicked by GABAergic, cholinergic, serotonergic or dopaminergic agonists or antagonists nor by the neurotoxins N-methyl-D,L-aspartic acid and ibotenic acid. Furthermore, dihydrokainic acid and allo-kainic acid, which have low affinity for KA receptors, also failed to stimulate amino acid efflux.

These experiments provide physiologic evidence of a presynaptic localization of KA receptors, which has previously been documented by ligand-binding techniques. They also indicate that KA can induce the release of excitatory amino acids independent of impulse flow and are consistent with previous findings that transection of excitatory afferents alone is insufficient for protection against the neurotoxic effects of kainic acid but that actual degeneration of the excitatory afferents is required. (Supported by USPHS Grants NS 13584 and MH 00125 and USPHS Fellowship NS 06798 to JWF).

251.4 ACIDIC AMINO ACID RECEPTOR POPULATIONS IN SYNAPTIC MEMBRANES: SEPARATION OF Cl^-/Ca^{2+}-DEPENDENT AND -INDEPENDENT SITES BY FREEZING. G.E. Fagg*, E.E. Mena, D.T. Monaghan* and C.W. Cotman (SPON: J.W. Dailey). Dept. Psychobiology, Univ. of California, Irvine, CA 92717.

Recently, we demonstrated the presence of two distinct populations of Na^+-independent L-glutamate binding sites in SPMs: (1) dependent on Cl^- and Ca^{2+} ions and potently inhibited (K_I = 5 µM) by the L-isomer of 2-amino-4-phosphonobutyrate (APB, a glutamate analog), and (2) Cl^- and Ca^{2+}-independent and insensitive to low micromolar concentrations of APB. The present study was conducted in order to evaluate the effects of freezing on SPM-located L-glutamate binding sites.

When assayed at 30°C in 50 mM Tris-acetate buffer (pH 7.2), the level of L-[^{3}H]glutamate (50 nM) binding in frozen and thawed SPMs was greater than 2-fold higher than in matched fresh SPMs. Binding in frozen SPMs was optimal at pH 6.8-7.2, showed little temperature dependence (30°C vs. 0°C), and exhibited saturation kinetics, with a predominant component of K_D 0.5 µM (determined at 30°C). In contrast to fresh SPMs, neither 5 mM Cl^- nor 2.5 mM Ca^{2+} enhanced L-glutamate (50 nM) binding to frozen SPMs, and other ions (Na^+, K^+, Mg^{2+}) similarly exerted little effect.

Scatchard analyses of the inhibition of L-glutamate binding to frozen SPMs by DL-APB (1 µM - 10 mM) yielded an apparent K_I of 2 mM; this value was similar both in the presence or absence of Cl^- and Ca^{2+}. Similar analyses using fresh SPMs (in the presence of Cl^- and Ca^{2+}) revealed both this low affinity site and the previously reported high affinity (K_I = 5 µM) component. In contrast to Cl^-/Ca^{2+}-dependent L-glutamate binding in fresh SPMs, APB was a less potent inhibitor in frozen SPMs than the longer chain homolog, 2-amino-5-phosphonovalerate. Additional pharmacological experiments using frozen SPMs indicated that the L isomers of glutamate and aspartate were potent displacers of L-glutamate binding, and their enantiomers were less effective; L-serine-O-sulfate, L-homocysteate and ibotenate were moderately effective; and quisqualate, N-methyl-aspartate and kainate were weaker displacers.

These data indicate that freezing selectively destroys the Cl^-/Ca^{2+}-stimulated population of L-glutamate binding sites in SPMs, and that the freezing-resistant sites may be equated with the Cl^-/Ca^{2+}-independent population in fresh SPMs. Detailed pharmacological studies of these sites may aid elucidation of the classes of excitatory amino acid receptors previously defined using electrophysiological techniques.

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251.5 ACIDIC AMINO ACID RECEPTOR POPULATIONS IN SYNAPTIC MEMBRANES: REGULATION BY Cl^- and Ca^{2+} ions. E. E. Mena, G. E. Fagg*, D. T. Monaghan* and C. W. Cotman. Department of Psychobiology, University of California, Irvine, CA. 92717

Recent results from our laboratory demonstrate that Cl^- and Ca^{2+} act in concert to reveal a distinct class of L-Glu binding sites characterized by their sensitivity to the phosphonic acid analog L-2-amino-4-phosphonobutyric acid (APB). The present study was conducted to evaluate both the anion specificity of this response and the interaction between Cl^- and Ca^{2+}.

Several anions were examined over a range of 1-100 mM for their ability to enhance L-Glu binding to SPMs. Of the ions tested, Br^- was the most effective, increasing L-Glu binding 2.6 fold with a maximal effect at 10mM. Other effective anions were Cl^- (2.43 fold), NO_3^- (2.33 fold) and formate (1.25 fold). All anions had their maximal effect at approximately 10mM. F^-, HCO_3^-, ClO_4^- and propionate were ineffective whereas SO_4^{2-} and PO_4^{3-} were inhibitory at concentrations >10mM. The anion specificity of this binding site is similiar to that for the membrane Cl^- channel, suggesting that these two may be linked.

The interrelationship of Ca^{2+} and Cl^- in enhancing L-Glu binding was investigated by Scatchard analysis of saturation curves for each of these ions (50nM ^3H L-Glu). In the absence of Cl^-, Ca^{2+} has little effect on L-Glu binding. Increasing the Cl^- concentration decreases the K_D and increases the B_{max} of Ca^{2+}. Hence, as Cl^- is increased from 5mM to 42mM the K_D for Ca^{2+} progressively decreased from 174 µM to 29 µM, and the B_{max} increased from 2.34 pmol/mg to 3.91 pmol/mg. On the other hand, Scatchard analyses with respect to Cl^- indicated that increasing Ca^{2+} levels increases the B_{max} for Cl^- without affecting the K_D. Thus, in the presence of 5mM EGTA the K_D for Cl^- is 3.84 mM and the B_{max} is 0.92 pmol/mg. In the presence of 1 mM Ca^{2+} the K_D is 3.67 mM while the B_{max} increases to 3.42 pM/mg.

The mechanism of action of Ca^{2+} and Cl^- was investigated by incubating SPMs in 50mM Tris-HCl (pH 7.4) containing 0.5mM Ca^{2+} for various lengths of time; Ca^{2+} was subsequently removed by the addition of 5 mM EGTA with complete reversal of the effect of this ion. In addition, EGTA added 2 min. prior to the termination of the assay caused a large reduction of Cl^-/Ca^{2+} binding. Furthermore, membranes thta were incubated with Ca^{2+} and Cl^- and washed free of both of these ions prior to assay in Tris-acetate buffer contained identical levels of binding as in untreated membranes. The demonstration that the effects of Cl^- and Ca^{2+} are reversible indicate that these ions interact ionically with the receptor molecule to reveal the APB-sensitive class of L-Glu binding sites. Supported by grants from the NIH (NS08597) and the State of CA. Dept. of Health Services (GA80-00132).

251.6 QUISQUALATE AND IBOTENATE DISTINGUISH TWO BINDING SITES FOR L-[^3H] GLUTAMATE ON HIPPOCAMPAL SYNAPTIC MEMBRANES IN THE ABSENCE OF SODIUM. Linda L. Werling and J. Victor Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Considerable evidence suggests that glutamate serves as an excitatory neurotransmitter in the rat hippocampal formation. We have therefore sought to identify and characterize glutamate receptors in binding studies on hippocampal synaptic membranes. The glutamate analogues, quisqualate and ibotenate, are believed to depolarize CNS neurons by interacting primarily with different excitatory amino acid receptors. We have now used these analogues to distinguish hippocampal binding sites for L-[^3H]glutamate. The fraction of bound radioligand that was displaceable by 5 µM quisqualate is referred to as GLU A binding. That which persisted in the presence of 5 µM quisqualate, but was displaceable by 100 µM ibotenate, is referred to as GLU B binding.

Freshly-prepared hippocampal synaptic membranes were washed four times with water to remove endogenous ligands and were incubated with L-[^3H]glutamate (35 Ci/mmol) in a 1.4 ml volume of tris-HCl buffer without inorganic cations. GLU A binding equilibrated within 5 min, whereas GLU B binding plateaued between 2 and 10 min of incubation and then increased to a maximum in 60 min. Both were greatest around 38°C and at a slightly acidic pH. Saturation curves were best fit by single exponentials which yielded K_D values of 190 nM (GLU A) and 1.2 µM (GLU B), with corresponding B_{max} values of 37 and 58 pmol/mg protein. Hill coefficients were not significantly different from unity, indicating the absence of co-operative interactions. Rapid freezing of the unwashed membranes, followed by storage at -26°C for 1-24 days, rapid thawing, and washing markedly reduced GLU A binding, but approximately tripled GLU B binding. The GLU A site bound L-glutamate with much higher affinity than L-aspartate or D-glutamate. In general, amino acid excitants with longer carbon chains most potently displaced L-[^3H]glutamate from this site, and all antagonists tested exhibited moderate-to-high affinity. The GLU B site was also stereospecific for L-glutamate, but bound L-aspartate with equally high affinity. Amino acid excitants with shorter carbon chains most potently displaced L-[^3H]glutamate from this site, and all antagonists tested exhibited very low affinity.

These results are compatible with the hypothesis of multiple glutamate receptors in the rat hippocampal formation. Some properties of the GLU B binding site resemble those of the postsynaptic receptor at synapses made by axons of hippocampal CA3 pyramidal cells. (Supported by NIH grant NS 16064.)

251.7 EXCITOTOXICITY: STRUCTURE-ACTIVITY RELATIONSHIPS OF PYRIDINE AND PIPERIDINE CARBOXYLIC ACIDS. Alan C. Foster and Robert Schwarcz. Maryland Psychiatric Research Center, Univ. of Maryland, Baltimore, MD 21228.

The dicarboxylic acids of pyridine and piperidine are conformationally restricted analogues of the excitatory amino acids glutamate and aspartate. Quinolinic acid (2,3-pyridine dicarboxylate; QUIN) is a potent neurotoxin which causes axon-sparing lesions after intracerebral injection similar to those produced by kainate and ibotenate (Schwarcz and Whetsell, this meeting).

Injection of cis-2,3-piperidine dicarboxylate (2,3-PDA; 600 nmol in 1 µL, pH 7.4; nembutal anaesthesia) into the striatum or hippocampus of male rats resulted in extensive, but circumscribed, neuronal degeneration. The "distant" neuronal loss characteristic of intracerebral kainate application was not observed. Comparison of choline acetyl transferase activities following intrastriatal injection at a dose of 600 nmol indicated that 2,3-PDA was slightly less potent than QUIN (46±8%, 13±4%, respectively of contralateral side, N=5). In the same manner as QUIN, the neurotoxic action of 2,3-PDA was axon-sparing since tyrosine hydroxylase activity was unchanged in injected striata (103±8% of contralateral side, N=5). Two further similarities between 2,3-PDA and QUIN were (1) the relative sparing of hippocampal granule cells after injection of 30-60 nmol of the amino acids and (2) a lack of neurotoxic effects after injection (120 nmol) into the striata of 7 day old rat pups.

Attempts were made to correlate the in vivo actions of 2,3-PDA and QUIN with their affinity for acidic amino acid receptors and glutamate transport sites. At a concentration of 1 mM, both compounds were weak (<30%) inhibitors of either L-^3H-glutamate or ^3H-kainate binding to striatal membranes and of Na^+-dependent glutamate uptake in striatal P_2 suspensions.

As assessed at the light microscopic level, 600 nmol of 3,4- or 2,6-pyridine dicarboxylate had weak neurotoxic effects in the striatum; at the same dose the following compounds were inactive: 2,4-, 2,5- and 3,5-pyridine dicarboxylate, 2,5- and 2,6-PDA, nicotinic, picolinic and pipecolic acids. Also, none of the compounds caused >30% inhibition of glutamate binding, kainate binding or Na^+-dependent glutamate uptake in striatal tissue at a concentration of 1 mM.

These findings suggest that 2,3-PDA and QUIN exert their effects through similar mechanisms, which do not primarily involve known acidic amino acid binding sites or Na^+-dependent glutamate transport systems. The failure of other pyridine- and piperidine dicarboxylates to show any appreciable neurotoxicity indicates that the receptor(s) mediating the effects of 2,3-PDA and QUIN has strict structural requirements for activation.
This work was supported by USPHS grant NS-16941.

251.8 ATTENUATION OF SPIKES, GLUTAMATE-ACTION AND EPSPs BY INTRACELLULAR QX222 IN HIPPOCAMPAL CA 1 NEURONS. E. Puil[1] and P. L. Carlen. Departments of Medicine and Physiology, University of Toronto, Addiction Research Foundation and Playfair Neuroscience Unit, Toronto Western Hospital and [1]Departments of Anaesthesia and Pharmacology, Faculty of Medicine, University of British Columbia, Vancouver, B.C., Canada.

Quaternary analogues of lidocaine such as QX222, which are relatively slow to traverse lipid membranes, prevent conduction of nerve impulses by blocking voltage-dependent Na^+ channels from the cytoplasmic side of the axolemma. Following iontophoretic injection of QX222 (.05 to 1.0 M; 0.5 to 2.5 nA for 30-300s) into CA 1 neurons of in vitro hippocampal slices of guinea pig brain, we observed a strong depression of spontaneous, electrically-(current injection) or orthodromically-evoked action potentials. These effects were characterized by a reduction in the rate of rise and amplitude of spikes and were not usually accompanied by marked changes in resting membrane potential, although membrane conductance tended to diminish with prolonged applications of QX222. The current threshold for electrically-evoked spikes increased markedly which also reflected the gross impairment of Na^+-electrogenesis. The reduction of spike height by QX222 may be partly attributable to enhanced inactivation of Na^+-channels because brief depolarizing pulses preceded by strong hyperpolarization (imposed by current injection) which likely would remove sodium inactivation, elicited action potentials at a lower threshold and of considerably larger amplitude than in the absence of such hyperpolarization. When spikes were abolished by QX222, the depolarization evoked with application of S-glutamate by pressure-ejection from an extracellular micropipette was decreased or even blocked completely in 17 neurons. This reproducible blockade was surmountable by administering larger amounts of S-glutamate. An interesting finding is that EPSPs (evoked by stimulation of strata oriens or radiatum) were reduced in a similar manner by intracellular QX222. These data suggest that 1) voltage-dependent Na^+ channels in CA 1 neurons can be blocked by QX222 applied intracellularly, and 2) QX222 apparently interferes with the functions of the inner end of similar Na^+ channels activated by glutamate-receptor interaction or by receptor interactions with the neurotransmitter(s) associated with certain EPSPs in CA 1 neurons.
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251.9 EFFECTS OF AMINO ACID AGONISTS AND FOLATES ON ELECTROPHYSIOLOGICAL ACTIVITY IN THE PREPYRIFORM CORTEX SLICE. S. L. Rubinstein*, D. J. Braitman, and C. R. Auker. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

We previously reported (Hori et al., Cell. Mol. Neurobiol. 1:115, 1981) that neither glutamate nor aspartate appears to be the neurotransmitter at the terminal synapses of the lateral olfactory tract (LOT) whereas the nonendogenous compounds kainic acid (KA) and N-methyl-dl-aspartate (NMA) exhibit a pharmacological profile similar to that of the natural transmitter. In a continuing investigation of the identity of the endogenous neurotransmitter at the terminal synapses of the LOT, we examined the actions of folic acid (pteroyl-L-glutamic acid) and its derivatives (methyltetrahydrofolate and formyltetrahydrofolate) and of quinolinic acid (QUIN) on field potentials evoked by stimulation of the LOT in the in vitro prepyriform cortex (PPC) slice. Methyltetrahydrofolate, a naturally occurring substance in cerebral spinal fluid, has been reported to be a potent competitor for KA-binding sites (Ruck et al., Nature 287:852, 1980). QUIN is an endogenous pyridine dicarboxylic acid that excites amino acid receptors in rat cerebral cortex (Stone and Perkins, Eur. J. Pharm. 72:411, 1981). We compared the actions of these compounds to those of agonists typically used to characterize amino acid receptors, i.e., quisqualic acid (QUIS), NMA, and KA.

Field potentials were recorded from the pial surface of submerged, perfused tangential slices of rat PPC (300-450 μ). These field potentials typically consisted of a slow wave component representing the population excitatory postsynaptic potential (EPSP) and a population spike (PS) that reflects synchronous multineuronal discharge. Bath perfusion of KA in concentrations $\geq 10^{-6}$ M resulted in a reversible decrease in the amplitude of the EPSP and a reversible increase in the amplitude and width of the PS. QUIS and NMA reduced the EPSP and increased the PS at 5×10^{-5} M. At 10^{-5} M, QUIS decreased the amplitude of the EPSP without affecting PS size whereas NMA increased asynchronous spiking on the late portion of the field potential but had no effect on the size of the EPSP or PS. These effects demonstrate an excitatory action of these compounds on PPC with an order of potency of KA > QUIS ≥ NMA.

QUIN had no consistent effect on the LOT-stimulated field potential at concentrations $\leq 10^{-4}$ M. At 10^{-3} M it rapidly abolished the EPSP and PS, with little or no recovery. Late asynchronous spiking, similar to that seen with NMA, occurred in some experiments. Thus, QUIN appears to be toxic to PPC while its role as a receptor agonist remains unclear. In contrast to all other agonists tested, the folates had virtually no effect on LOT-stimulated EPSP or PS nor did they antagonize the action of KA. It appears unlikely that the folates are endogenous ligands for KA since they did not reproduce or block the action of KA even in concentrations as high as 5×10^{-3} M. We are presently investigating the effects of amino acid antagonists on the actions of QUIN and folates in order to further characterize these compounds and to compare them to the endogenous transmitter released by LOT stimulation.

251.10 EFFECTS OF EXCITATORY AMINO ACIDS ON SPONTANEOUS LOCOMOTOR ACTIVITY AFTER MICROINJECTION INTO THE NUCLEUS ACCUMBENS: POSSIBLE DOPAMINERGIC MECHANISM. B. A. Donzanti and N. J. Uretsky. Division of Pharmacology, College of Pharmacy, Ohio State University, Columbus, OH 43210.

In previous studies, we have found that D-glutamic acid (100 μg), when injected directly into the nucleus accumbens, produced an increase in spontaneous locomotor activity. In contrast, L-glutamic acid, at doses up to 200 μg, was inactive as a stimulant of this behavior. In the present experiments, we have studied the effects of various excitatory amino acids on the spontaneous locomotor activity of rats following bilateral injection of these compounds into the nucleus accumbens. Kainic acid (15-125 ng) and quisqualic acid (0.15-5 μg), compounds structurally related to glutamate, produced significant dose-dependent increases in locomotor activity. The peak stimulation after kainic acid occurred at a dose of 125 ng; the reduction in motor activity at higher doses (0.25-0.5 μg) was associated with tonic/clonic convulsive episodes. Although quisqualic acid at higher doses (2.5-10 μg) did not produce convulsions, it did at times elicit a characteristic "praying" response, which produced only a small inhibition of motor activity. Surprisingly, the administration of N-methyl-DL-aspartic acid did not produce an increase in locomotor activity at any dose tested but did elicit convulsions at 5 μg. Drugs that activate dopamine receptors in the nucleus accumbens have been shown to markedly stimulate locomotor activity. Therefore, we have investigated whether the effects of kainic acid and quisqualic acid in the nucleus accumbens are mediated by a dopaminergic mechanism. The stimulation of locomotor activity by both kainic acid (31 ng) and quisqualic acid (2.5 μg) was inhibited (84% and 73%, respectively), by pretreatment with haloperidol (0.8 mg/kg, i.p.), a dopamine receptor blocking agent. In addition, the effects of kainic acid (31 ng) were markedly inhibited (95%) by reserpine (5 mg/kg, i.p.), which depletes dopamine stores and (70%) by fluphenazine (2.5 μg), a dopamine receptor blocking agent, injected directly into the nucleus accumbens. This latter observation suggests that dopamine receptors within the nucleus accumbens mediate the stimulation of motor activity. In contrast to its effects on motor activity, haloperidol did not alter the frequency of convulsions produced by high doses of kainic acid. These results indicate that stimulation of locomotor activity produced by excitatory amino acids in the nucleus accumbens is mediated by dopaminergic mechanisms.

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251.11 CHARACTERIZATION OF DIFFERENT TYPES OF EXCITATORY AMINO ACID RECEPTORS IN THE HIPPOCAMPUS: II. BIOCHEMICAL STUDIES. M. Baudry, E. Smith*, L. Fagni*, and G. Lynch, Dept. of Psychobiology, University of California, Irvine, CA 92717.

Electrophysiological studies have indicated the existence of multiple receptors for excitatory amino acids. Similarly, various classes of receptors have been detected by measuring the stimulation of Na fluxes elicited in brain slices by such compounds. On the other hand, ligand binding studies have not provided an unequivocal demonstration of the association of binding sites for excitatory amino acids to physiologically relevant receptors. In the present study we compared the characteristics of glutamate receptors obtained by using these three different approaches applied to the same brain structure, the hippocampus.

^{3}H-Glutamate binding to hippocampal crude synaptic membranes was determined as previously described (Baudry and Lynch, J. Neurochem., 1981, 36, 811-820). Glutamate stimulation of ^{22}Na fluxes in hippocampal slices was measured according to Luini et al. (P.N.A.S., 1981, 78, 3250-3254). The properties of the receptors involved in the stimulation of ^{22}Na flux were found to be very similar to those of the receptors responsible for the amino acid-induced depolarization of pyramidal cells. Thus, dose-responses for various agonists were identical on both measures; the order of potency for a variety of amino acids were remarkably similar and the effects of several antagonists were also comparable. In addition, L-glutamate stimulation of Na fluxes exhibited a desensitization similar to that found with the electrophysiological approach. On the other hand, the pharmacology of the glutamate binding site was very different from that of the site assayed by the glutamate-stimulation of Na flux, as has been noted by others. However, drugs which block synaptic transmission in CA1 were found to inhibit ^{3}H glutamate binding, while several agonists which exhibit desensitization did not interfere with the binding.

These results point to the existence of at least four types of receptors for excitatory amino acids and suggest ways to study their relative localizations and functions: 1) a synaptic receptor (tentatively defined as a G_1 receptor) stimulated by D,L-homocysteic acid and labeled by ^{3}H-glutamate, 2) an extrasynaptic glutamate receptor (defined as a G_2 receptor) stimulated by L-glutamate, 3) an N-methyl aspartate extrasynaptic receptor and 4) a Kainate extrasynaptic receptor. Further, they indicate the utility of ^{3}H-glutamate binding for studies of postsynaptic receptors in hippocampus.

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251.12 CHARACTERIZATION OF DIFFERENT TYPES OF EXCITATORY AMINO ACID RECEPTORS IN THE HIPPOCAMPUS: I. ELECTROPHYSIOLOGICAL STUDIES L. Fagni*, M. Baudry, G. Lynch, Department of Psychobiology, University of California, Irvine, California 92717.

Glutamate is a putative neurotransmitter in the mammalian CNS. In the hippocampus, it is thought to be used by the perforant path and the Schaffer-commissural pathway. A major difficulty in establishing such a role is the existence of multiple acidic amino acid receptors and the lack of specific glutamate agonists and antagonists. Desensitization of glutamate receptors has been shown to occur in several invertebrate preparations and has been used to differentiate synaptic from non-synaptic receptors. In the present study, we used a similar approach, in conjunction with pharmacological studies, to differentiate various types of glutamate receptors and to investigate their possible participation in synaptic transmission.

Experiments were performed on rat hippocampal slices which were continuously perfused with medium to which drugs could be briefly added. The decrease in the orthodromic dendritic potentials elicited by stimulation of the Schaffer-commissural pathway or in the amplitude of the antidromic responses of CA1 pyramidal cells was measured to quantify the agonistic or antagonistic properties of the tested drugs. A first application of L-glutamate (LG) (1 mM) for 5 minutes resulted in a marked, reversible decrease of both responses, whereas successive applications of the same concentration of LG were less and less effective. However synaptic transmission was not decreased during the period in which pyramidal cells were totally unresponsive to LG. A pronounced desensitization was found also with N-methyl-DL-aspartate (NMA 0.02 mM) but not with D,L-homocysteate (DLH 0.05 mM) or kainate (K 0.01 mM). D,L-α-aminoadipate (6mM) blocked the orthodromic responses, as well as the excitatory responses to DLH and to NMA, but not those to LG nor to K.

These results suggest the existence of four types of receptors which can be differentiated on the basis of desensitization and their respective antagonist specificities: two 'desensitizable' extrasynaptic receptors - a LG receptor and a NMA receptor - and two 'non-desensitizable' receptors - a DLH synaptic receptor and a K extrasynaptic receptor.

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251.13 ELECTROPHYSIOLOGICAL CHARACTERIZATION IN THE HIPPOCAMPUS OF SERINE-O-SULFATE AND SERINE-O-PHOSPHATE: TWO NEW ACIDIC AMINO ACID ANALOGUES. Alan H. Ganong* and Carl W. Cotman. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

Recent experiments have identified 2-amino-4-phosphonobutyrate (APB) as a potent blocker of synaptic responses in three putative excitatory amino acid CNS pathways: the lateral perforant path input to dentate gyrus in the rat hippocampus; the lateral olfactory tract in rat prepyriform cortex; and the fast component of the evoked ventral root potential in frog and rat spinal cord. Ligand binding studies in this laboratory have demonstrated that the APB analogues serine-O-sulfate and serine-O-phosphate compete for glutamate (Glu) and APB binding sites in synaptic membranes; further, serine-O-phosphate blocks the lateral perforant path synaptic response. In this study we describe the agonist/antagonist interactions of these and other amino acid analogues in the lateral perforant path zone of the dentate gyrus.

Kainate (KA) applied by ionophoresis to the lateral perforant path zone resulted in relatively long duration, large amplitude negative deflections in the extracellular DC potential (focal potential). 2-amino-5-phosphonovalerate (APV), serine-O-phosphate, and APB were equipotent in inhibiting KA focal potentials; moderate ionophoretic applications of these compounds partially and reversibly blocked KA focal potentials. N-methyl-D-aspartate (NMDA) focal potentials could be elicited with relatively small ejection currents. APV completely blocked NMDA responses. Serine-O-phosphate was also a potent NMDA antagonist although less potent than APV. APB was less effective against NMDA responses than serine-O-phosphate. Glutamate and serine-O-sulfate behaved similarly in these experiments, both in their depolarizing action and in regard to the ability of antagonists to block Glu or serine-O-sulfate focal potentials. Glu and serine-O-sulfate required more current than KA or NMDA to produce depolarizations of equivalent amplitude. APV was a moderately potent blocker for Glu depolarizations and was somewhat more effective against serine-O-sulfate depolarization. However, serine-O-phosphate and APB were poor blockers of Glu or serine-O-sulfate focal potentials.

The present results show that APB and serine-O-phosphate selectively antagonize NMDA and KA depolarizations, but not Glu or serine-O-sulphate responses. The mechanism by which these compounds block the lateral perforant path (and other) synaptic response(s) therefore remains unexplained since they are ineffective against applied Glu, the presumed transmitter in this system. (Supported by NS 08957).

251.14 SENSITIVITY OF HIPPOCAMPAL NEURONS TO GLUTAMATE AGONISTS AND ANTAGONISTS. John J. Hablitz, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030.

Identification of receptors for amino acid neurontransmitters has been enhanced by the development of glutamate (GLU) agonists and antagonists. Although it generally has been assumed that all GLU agonists produce excitation via a depolarization associated with an increase in membrane conductance, this has never been directly shown. To better understand the action of these agents on cortical neurons, their effects on the membrane potential and input resistance (Rin) of CA1 neurons has been examined.

Intracellular recordings were obtained from the CA1 region of guinea pig hippocampal slices while agonists were applied via double barreled iontophoresis electrodes. One barrel always contained GLU, while the other contained either L-aspartate (ASP), DL-homocysteic acid (DLH), N-methyl-D-aspartic acid (NMA), kainic acid (KA), or quisqualic acid (QUIS) (all 0.3 M pH=8) in the other. The antagonists DL-aminoadipic acid (DLAA), L-glutamic acid diethylester (GDEE), and 2-amino-4 phosphonobutyric acid (APB) were bath applied at concentrations of 1-5mM.

Depolarization and cellular firing resulted from application of all agonists. When applied with low ejection currents (1-6nA) for long periods (2-10 sec) NMA and DLH produced apparent increases in Rin (10-45%). These increases appeared to be due to a direct transmitter action and not membrane rectification properties. The other agonists (and NMA and DLH at higher doses) produced decreases (20-90%) in Rin. GLU and ASP were approximately equal in potency but less potent than DLH, KA, NMA, and QUIS. Exact determination of potency was complicated by the fact that responses to GLU and ASP were more rapid in onset and decayed faster than responses to other agonists, and the magnitude of the conductance change varied during the response. The relative potencies of agonist compounds thus varied according to the response measure used. Furthermore, higher doses of the agonists induced extracellular DC shifts which distorted intracellular measurements. These extracellular shifts, which persisted when action potential generation was blocked by TTX and Mn2+, also caused long-lasting changes in neuronal responsiveness. Dose response curves measured in individual neurons before and after bath application of GDEE and DLAA were not significantly different. APB at 5 mM decreased responses to the agents tested (GLU and NMA). The high concentration of antagonists employed in these experiments contrast sharply with studies of inhibitory transmitter systems where antagonists are effective when bath applied at low micomolar levels. Further study with recently developed more potent GLU antagonists may reconcile these differences.

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251.15 AMINO ACID BENZYL ESTERS: INHIBITORS OF BRAIN MONOAMINE OXIDASE. A. Schurr and B. M. Rigor. Dept. of Anesthesiology, University of Louisville School of Medicine, Louisville, KY 40292

Studying the mechanism of action of plasma amine oxidase (PAO), Maycock and his colleagues (Maycock, A.L., Suva, R.H. and Abeles, R. H., J. Amer. Chem. Soc. 97: 5613, 1975) suggested that esters with sufficiently good leaving groups can undergo elimination reactions to form ketenes. Generation of a reactive ketene species at the enzyme's active site is leading to enzyme inactivation.

Glycine benzyl ester has a good leaving group and has been found to be a strong irreversible inhibitor of PAO. Glycine ethyl ester has a poor leaving group and does not inactivate the enzyme. Nevertheless, both compounds compete with each other for the same site on the enzyme molecule.

Monoamine oxidase (MAO), an enzyme of the outer mitochondrial membrane, is, like PAO, catalyzing the oxidative deamination of monoamines. The enzyme activity of rat brain mitochondria is inhibited by a variety of amino acid benzyl esters but not by amino acid methyl, ethyl or butyl esters. Unlike PAO, MAO inhibition by glycine benzyl ester is reversible. Both 5-hydroxytryptamine (5-HT) deamination (type A activity) and 2-phenylethylamine (PEA) deamination (type B activity) are inhibited by this inhibitor. However, while the type B activity is highly sensitive to this inhibitor (50% inhibition at 10^{-5} M), type A activity is more resistant to the action of this ester (50% inhibition at 10^{-3} M). Despite this difference in sensitivity, the inhibition of both activities by glycine benzyl ester is competitive and occurs instantly.

Other amino acid benzyl esters, although not a strong inhibitors as glycine benzyl ester, demonstrate specific B/A ratios of inhibition (the inhibitor concentration needed for 50% inhibition of PEA deamination/the inhibitor concentration needed for 50% inhibition of 5-HT deamination). While the B/A ratio for glycine benzyl ester is 0.01, that for proline benzyl ester is 0.001 and the B/A ratio of inhibition for alanine benzyl ester is only 0.3.

Experiments which are aimed toward better understanding of the mode of action of these MAO inhibitors, their selectivity, and specificity in vivo are under way.

251.16 MEASUREMENT OF EXCITATORY AMINO ACID - INDUCED DEPOLARIZATION IN ISOLATED - RESEALED SYNAPTIC PLASMA MEMBRANES. H. H. Chang*, E. K. Michaelis, and S. Roy*, Dept. of Human Development and Ctr. for Biomedical Research, Univ. of Kansas, Lawrence, Ks 66045

L-Glutamate (L-Glu) and L-Aspartate (L-Asp) cause transient membrane conductance for Na^+ in vertebrate neurons leading to depolarization of these cells. L-Glu, L-Asp, L-cysteine sulfinic, and Kainic acid (KA) have been shown to enhance Na^+ fluxes across the vesicular membrane of resealed synaptic plasma membrane vesicles (Chang & Michaelis, JBC 255, 2411, 1980; JBC 256, 10084, 1981). The flux of Na^+ across the synaptic membrance induced by L-Glu was electrogenic in nature, i.e., it induced membrane depolarization (Chang & Michaelis, BBA, in press). The electrogenicity of the Na^+ flux stimulated by L-Glu was demonstrated by measuring the distribution of the lipophilic anion [^{35}S] thiocyanate (SCN-) into synaptic membrane vesicles incubated in a NaCl or Na_2SO_4 medium. Based on the SCN- distribution, it was calculated that 10μM L-Glu induced an average membrane potential change of +13 mV.

Other neuroexcitatory amino acids and amino acid analogs (D-Glu, L-Asp, L-cysteine sulfinate, KA, ibotenate, quisqualate, N-methyl-D-aspartate, and D,L-homocysteate) also increased SCN- accumulation in synaptic membrane vesicles. The stimulation of SCN- accumulation by L-Glu, D-Glu, KA, and N-methyl-D-aspartate (NMDA) was a dose-dependent process with maximal stimulation produced by these agents at concentrations ranging from 1-50μM. Such observations indicated activation by L-Glu and its analogs of excitatory amino acid receptor-ion channel complexes in synaptic membranes. However, since neuropharmacologic and biochemical studies have previously shown that D-Glu and NMDA interact with the same receptors while L-Glu and KA activate distinct receptors, the specificity of the synaptic membrane receptor sites involved in the depolarization response was explored further. L-Glutamate -induced SCN- accumulation was strongly antagonized by 100μM glutamate diethylester and D,L-α-methyl glutamate, and more weakly blocked by 100μM 2-amino-4-phosphono butyric acid (2-APB) and 2-amino-3-phosphono propionic acid (2-APP). Both 2-APB and 2-APP exhibited agonist-like activity. The KA-induced stimulation of SCN- influx was also antagonized by 2-APB. Furthermore, the KA and the L-Glu-enhanced SCN- accumulation did no represent additive responses, whereas the L-Glu and NMDA effects on SCN- accumulation were additive. It thus appears that L-Glu and KA receptors in synaptic membranes interact to produce depolarization, whereas the NMDA and L-Glu receptors function independently. (Supported by grants AA 04732 from NIAAA and DAAG 29-79-C-0156 from US Army Res. Office.)

251.17 EFFECTS OF AMINO ACID ANTAGONISTS ON EPILEPTIFORM BURSTS INDUCED BY FOLIC ACID, KAINIC ACID AND N-METHYL-D,L-ASPARTIC ACID, IN VITRO. D.B. Clifford, E.W. Lothman and J.A. Ferrendelli. Dept. of Neurology, Washington University School of Medicine, St. Louis, MO 63110.

Recent studies of Ruck et al. (Nature 287: 852-853, 1980) suggested that in cerebellar membranes methyltetrahydrofolic acid (MTHF) competes for binding of the powerful neurotoxin, kainic acid (KA), suggesting the possibility of an epileptogenic role of folate compounds in the brain. Olney et al. have found that injections of folates cause seizures clinically like those caused by KA, with similar neurotoxic properties (Nature 292: 165-167, 1981). Pteroyl-L-glutamic acid (PGA, folic acid) and folinic acid were much more neurotoxic than MTHF. We have studied the electrophysiologic effects of PGA, KA and analogues in hippocampal slices and found that spontaneous epileptiform activity is generated by folates, PGA being most potent, and that this activation is independent of metabolic roles of PGA (submitted). The possibility that folates are neuromodulators related to the excitatory neurotransmitters is under investigation.

Several lines of evidence favor the existence of at least three types of excitatory amino acid receptors, named for potent agonists N-methyl-D-aspartate (NMDA), glutamate (glu) or quisqualate and KA receptors. The pattern of response to amino acid antagonists is one means of differentiating these sites. D-alpha-aminoadipate (D-AA) has greater specificity for blocking NMDA effects, while L-glutamic acid diethylester (GDEE) seems more specific for glu effects. KA-induced changes have been resistant to both of these agents, although 2-amino-5-phosphonobutyrate (APB) has in some systems antagonized its effects. In this study, the effects of D, L-APB, L-GDEE, D- and D,L-AA, on spontaneous epileptiform bursting generated in guinea pig hippocampal slices, in vitro, by PGA 200 µM, KA 0.05-0.1 µM or NMDA 1-5 µM. After establishing epileptiform discharges in the slices, while recording in area CA3 with extracellular microelectrodes, the concentration of amino acid antagonist was increased in the perfusate. PGA bursting was blocked routinely by 0.5-1.0 mM APB, by 1/2 trials of DL-AA 3 mM but by only 1/5 2 mM, and never by GDEE. Kainate epileptiform activity was blocked routinely by 0.25-0.3 mM APB but not by GDEE or AA up to 4 mM. NMDA activity was blocked by 0.5-1 mM D-AA, 3 mM D,L-AA, 1-3 mM APB, and never by GDEE. Penicillin-induced epileptiform is not blocked by APB or AA.

The profile of activity of amino acid antagonists suggests that at least the PGA and KA are blocked in a similar manner, and this pattern is different from that of NMDA. This evidence supports the hypothesis that folates interact or modify activity at a KA site, although it in no way proves such an interaction.

251.18 EFFECTS OF LOW DOSES OF Co^{++}, Mg^{++} and Mn^{++} ON SYNAPTIC TRANSMISSION IN AMPHIBIAN SPINAL CORD. P.A. Smith (SPON: W.F. Dryden). Dept. of Pharmacology, Univ. of Alberta, Edmonton, Canada, T6G 2H7.

Divalent cations are known to antagonize responses generated at NMDA (N-methyl-D-aspartate) receptors by agonists such as N-methyl-D-L aspartic acid (NMDLA). (Watkins & Evans, A. Rev. Pharm. Tox., 21:165, 1981). In higher doses, divalent cations block neurotransmitter release. Sucrose gap recording was used to monitor synaptic transmission in amphibian sympathetic ganglia. Mn^{++} (1mM) was much more effective than 1mM Co^{++} or Mg^{++} in reducing neurotransmitter release, i.e. in reducing the amplitude of the postganglionic population action potential. The dorsal root - ventral root potential (DR-VRP), dorsal root-dorsal root potential (DR-DRP) and ventral root-dorsal root potential (VR-DRP) of the hemisected amphibian spinal cord were also examined by means of the sucrose gap technique. Mn^{++} (125 or 250µM) produced nonselective depression of all three of these synaptic responses, presumably by impairment of neurotransmitter release. On the other hand, Co^{++} (125µM) or Mg^{++} (250µM) produced depression of only the slow (polysynaptic) components of DR-VRP and DR-DRP. VR-DRP, which is also a polysynaptic response (Barker et al., J. Physiol., 245:537, 1975), was not reduced. Since synaptic transmission (VR-DRP) was preserved in the presence of Co^{++} and Mg^{++}, their observed effect on DR-DRP and DR-VRP is unlikely to result from impairment of neurotransmitter release. In spinal cords where syanptic transmission was blocked with 0.2µM tetrodotoxin, superfusion of 40µM NMDLA for 1-3 min produced depolarization of both ventral roots (VR: motoneurones) and primary afferents (DR: dorsal roots). Co^{++} and Mg^{++} (250µM) were much more effective than Mn^{++} (250µM) in antagonizing these responses. Responses of VR to glutamate (GLU: 1mM) or aspartate (ASP: 1mM) were not significantly reduced. It is suggested that the selective depression of the polysynaptic components DR-VRP and DR-DRP by Co^{++} and Mg^{++} is due to antagonism of postsynaptic NMDA receptors. Since responses to GLU and ASP were insensitive to 250µM doses of these divalent ions, it is possible that neither of these putative neurotransmitters is involved in synaptic activation of NMDA receptors for generation of the polysynaptic components of DR-DRP and DR-VRP. Also, since VR-DRP was insensitive to Co^{++} and Mg^{++}, it is unlikely that any of the neurotransmitters involved in this pathway exert their postsynaptic effects via NMDA receptors. This latter result is in agreement with previous data obtained with the "organic" NMDA receptor blocker, DL-α-aminoadipic acid (Padjen & Smith, Can. J. Physiol. Pharmacol., 58:692, 1980).

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251.19 ACTIONS OF KAINIC ACID AND DEPRESSANT DRUGS ON THE RELEASE OF D-[^{3}H]ASPARTATE IN BRAIN SLICES. S.J. Potashner and D. Gerard*. Dept. of Anatomy, Univ. of Conn. Hlth. Ctr., Farmington, CT 06032.

Kainic acid, a rigid analogue of glutamate, exhibits excitatory and sometimes convulsant properties when applied to brain tissues. In addition, concentrations of kainic acid above 1-2mM are neurotoxic. Although evidence suggests that kainic acid may act by potentiating the postsynaptic response to certain excitatory transmitters, it is not known if the toxin affects the synaptic release of transmitters. To investigate this possibility, a study was made of the actions of kainic acid on the release of D-[^{3}H]aspartate (D-ASP), a compound which is taken up and released by neurons presumed to use glutamate or aspartate as their transmitter.

Albino guinea pigs were stunned by a blow to the head and decapitated. The left and right striata were dissected and sliced with a Stadie-Riggs microtome. Tangential slices were also taken from the cerebral neocortex. Slices were 300-350µ thick and weighed 20-30mg. After preincubation (30 min) and incubation (45 min) with D-ASP (31nM, 1µCi) or [U-^{14}C]GABA (500nM, 0.2µCi), slices were superfused with isotope-free medium. Superfusate medium was counted to measure the release.

Electrical stimulation of the slices for 4 min during superfusion evoked a Ca^{++}-dependent release of D-ASP and GABA. Exposure of the slices to 10 and 100µM kainic acid for 30 min during the incubation period did not change the evoked release of GABA, but enlarged that of D-ASP 2X in neocortex and 1.6X in striatum. Similar levels of dihydrokainic acid, an analogue of kainic acid with little excitatory or toxic action, did not increase D-ASP release.

Attempts were made to block the action of kainic acid with baclofen and pentobarbital, drugs which depress the release of glutamate and aspartate. When present in both the incubation and superfusion media, baclofen (4µM) inhibited the evoked release of D-ASP by 67% and prevented the enhancement of the release above control levels usually produced by 100µM kainic acid. Pentobarbital (100µM) produced a smaller inhibition of D-ASP release (27-35%), but also blocked the action of kainic acid.

The data suggest that 10 and 100µM kainic acid may enhance the synaptic release of glutamate and aspartate. This action, which might play a role in kainate-induced seizures and neuropathology, is prevented by baclofen and pentobarbital. [Supported by the Conn. Chapter of the Committee to Combat Huntington's Disease].

251.20 DIFFERENTIAL RESPONSES OF HIPPOCAMPAL NEURONS TO CYCLIC ANALOGUES OF ACIDIC AMINO ACIDS. J.F. Koerner, L.E. Rathe*, and R.K. Freund*. Dept. of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455.

Cyclic analogues of acidic amino acids depolarize and evoke action potentials in many CNS neurons, but the number of classes of receptors mediating these responses and their biological roles are unknown. We have devised a rapid assay using extracellular recording which gives a reproducible measure of agonist activity for hippocampal neurons. A slice of rat hippocampus, submerged in superfusing medium, was exposed to bath-applied agonist at a threshold concentration for inhibiting the stimulus-evoked extracellular synaptic field potential of an excitatory pathway afferent to the neurons under investigation. The drug concentration was doubled every four minutes until synaptic activity was inhibited >70%. The results from the cumulative dose-response curve are presented as the IC_{50} for agonist-induced blockade. The method was applied to dentate granule cells (GC), CA1 pyramidal cells, and CA3 pyramidal cells by activating the medial perforant path, Schaffer collaterals, and mossy fibers, respectively. The results for kainic acid (KA), DL-trans-2,3-piperidine dicarboxylate (trans-2,3-PDA), and DL-trans-2,4-piperidine dicarboxylate (trans-2,4-PDA) are summarized in this table:

	IC_{50} (µm)			Relative Potency		
	KA	2,3	2,4	KA	2,3	2,4
GC	3.8	59	390	1	1	1
CA1	1.7	18	110	2.2	3.3	3.6
CA3	0.23	19	220	17	3.1	1.8

The data for KA were consistent with the range of effective concentrations and rank order previously obtained by extracellular and intracellular studies [Ryan & Cotman, Soc. Neurosci. Abstr., 4 (1978) 227; Robinson & Deadwyler, Brain Res., 222 (1981) 117]. KA was the most potent agonist for all three classes of neurons; trans-2,4-PDA the least potent. None of the drugs showed parallel rank order of potency for the three classes of neurons. Most notably, KA was seven times more potent for CA3 than CA1 while trans-2,3-PDA was of equal potency for the two classes of pyramidal cells. In contrast to trans-2,3-PDA, trans-2,4-PDA was consistently less potent for CA3 than CA1. The data suggest that these cyclic analogues of acidic amino acids may interact with different classes of receptors on hippocampal neurons. (Supported by the Minnesota Medical Foundation.)

251.21 ACTIONS OF KAINATE ON SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES. G.L. Collingridge, S.J. Kehl* and H. McLennan*. Department of Physiology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Injections of kainic acid can produce seizures and a pattern of neuronal destruction which are similar to those observed in limbic lobe epilepsy. In the hippocampus, the area most sensitive to these actions, the neurotoxicity of kainate requires intact excitatory innervations. We have reported that in hippocampal slices iontophoretically-applied kainate greatly potentiates the Schaffer collateral-evoked excitatory response of CA1 neurones (Collingridge & McLennan, Neurosci. Letts., 27: 31, 1981) and have now extended these observations.

Brief iontophoretic applications of kainic acid (10-60 sec, 0-100 nA) to cell body regions of the slice usually potentiated (20-500%) for long periods (15-60 min) the amplitude of the population spike recorded in CA1, CA3 or the dentate gyrus following stimulation of their major excitatory inputs, the Schaffer collaterals, the mossy fibres and the lateral or medial perforant path respectively. In CA1, where this effect was investigated further, similar potentiations were produced by the iontophoretic administration of kainate to the apical dendrites where the stimulated fibres terminated (stratum radiatum) or by superfusion of kainate (10^{-6}-10^{-4}M). With brief superfusions (approx. 1 min) the threshold dose for synaptic potentiation (lasting 15 min or longer) was about 10^{-6}M, while at 10^{-5}M kainate caused massive potentiation (up to 1300%) which usually lasted at least 45 min. Similar effects were obtained in slices from which the CA3 region had been surgically removed, implying that effects were not mediated via actions on CA3 cells.

Potentiation was associated with a reduction in the amplitude of the field EPSP but no change in the size of the presynaptic fibre volley recorded in stratum radiatum. The antidromic population spike evoked by stimulation in the alveus and recorded at the cell body region was little or not affected. If an orthodromic response was preceded 10-100 msec by an antidromic response, the amplitude of the orthodromic population spike was reduced. This inhibition was often initially decreased by kainate but recovered after 5-10 min and was then usually potentiated.

These data indicate that the ability of kainate to potentiate synaptic excitation does not appear attributable either to prolonged disinhibition or to an increased excitability at the soma of CA1 neurones. Synaptic potentiation may, however, result from kainate producing a long lasting depolarization in the dendrites.

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251.22 STRUCTURE-FUNCTION RELATIONSHIPS FOR GAMMA SUBSTITUTED GLUTAMATE ANALOGUES ON DENTATE GRANULE CELLS. R.K. Freund*, J.F. Koerner, and R.L. Johnson* (SPON: R.L. Purple). Depts. of Biochemistry and Medicinal Chemistry, Univ. of Minnesota, Minneapolis, MN 55455.

We compared the pharmacology of γ-substituted glutamate analogues on perforant path-granule cell synapses from medial and lateral entorhinal cortex in transverse slices of rat hippocampus. We previously demonstrated in CA1 that bath-applied agonists can be distinguished from antagonists by extracellular recording of synaptic field potentials [Koerner and Cotman, Brain Res., in press]. Extracellular signs of agonist activity include appearance of a population spike evoked by an initially subthreshold stimulus and characteristic anomalies in the inhibitory and kinetic behavior of the drug-treated slice. These agonist responses are consequences of cell depolarization and thus should be neuron-specific, whereas antagonist responses, reflecting differences in receptor type, would be expected to be pathway-specific. We now report the use of these methods in the perforant path, in which medial and lateral entorhinal cortical projections converge on common granule cells, allowing neuron- vs. pathway-specificity to be tested. D- and L-glutamate, L-2-amino-4(5-tetrazolyl)-butanoic acid [glutamate tetrazole], and D- and L-homocysteic acid all elicited extracellular signs of agonist activity. Each compound appeared equally potent for inhibiting field potentials recorded from the medial and lateral perforant paths. Structure-function comparisons suggest that the planar γ-carboxyl group of glutamate can be replaced with either a planar (tetrazolium) or tetrahedral (sulfonate) anionic group and retain agonist activity. Only L-2-amino-4-phosphonobutyric acid (L-APB) [Koerner and Cotman, Brain Res., 216 (1981), 192] and L-O-phosphoserine (L-OPS) [Foster, Fagg, Harris and Cotman, Brain Res., in press] have been identified as antagonists. Both were more potent inhibitors of the lateral than of the medial pathway; D-isomers of either compound were only very weakly inhibitory; both could distinguish two medial components, one of moderate sensitivity comprising 30% of the total response, and one insensitive to millimolar concentrations; and they were the only analogues tested in which the γ-substituent can exist as a dianion at physiological pH. Both DL-APB and L-OPS exhibited increasing potencies when the pH of the superfusing medium was increased in the range pH = 7.0-7.8. This pH dependency supports the structure-function evidence that the γ-dianion form is the active species, although it does not preclude the possibility that the ionization state of the receptor is involved to some extent. (Supported by the Minnesota Medical Foundation.)

251.23 THE ACTIONS OF NMDA ON CAT SOMATOSENSORY CORTEX CELLS IN VITRO. J.A. Flatman*, P.C. Schwindt*, W.E. Crill, and C.E. Stafstrom (SPON: D. Farrell). Depts. of Physiol. & Biophys., and Medicine, Univ. Wash. Sch. Med. and VA Med. Ctr., Seattle, WA 98195.

It has long been known that N-methyl-D-aspartate (NMDA) exerts as potent an excitatory action on mammalian neocortical cells as on neurons elsewhere in the CNS (Crawford, J.M. and Curtis, D.R., Br. J. Pharmac., 23:313, 1964). Receptors for NMDA are believed to be present on sub-synaptic membranes throughout the CNS. Although the actions of agents such as L-glutamate and ibotenate have been investigated on current-clamped somatosensory neocortical cells (Puil, E., Brain Res. Rev., 3:229, 1981), no similar study of NMDA has been reported until now.

Tissue slices were prepared from the pericruciate gyrus of pentobarbitone anesthetized cats and maintained in vitro (Stafstrom et al., Brain Res., 236:221, 1982). Intracellular recordings were made predominantly on large, layer V (Betz) cells and NMDA was applied by droplet (10-100 μM) to the slice surface or by iontophoresis (0.05 M) close to the cell impaled.

NMDA evoked a slow reversible depolarization accompanied by a decrease of membrane slope conductance (G_M) and stable action potential repetitive firing (RF). However, during large depolarizations induced by NMDA, RF failed and G_M increased greatly. Slow oscillations of membrane potential (E_M), on which bursts of spikes could be fired, appeared towards the end and after the period of RF. Recovery of E_M and G_M developed very slowly following massive NMDA applications and was often incomplete. Large increases of synaptic noise were seen following droplet applications. In the presence of tetrodotoxin (TTX), NMDA still evoked depolarizations with G_M decrease, and rhythmic slow depolarizing waves developed, particularly in the presence of 10mM TEA. These oscillations (showing pre- and spike potentials) were abolished in calcium-free, 2mM cobalt-containing solutions (which could totally abolish evoked synaptic potentials). These evoked calcium spikes were generated at more negative levels of E_M during the application of NMDA. In TTX-TEA-cobalt containing solutions, which block most voltage dependent currents, NMDA still depolarized neocortical cells. A bi-stable E_M state developed which reflects a negative resistance region on the I/V curve.

In conclusion, depolarizations with G_M decrease are typically evoked by NMDA, as are calcium spikes (under suitable conditions) and a negative resistance region of the I/V curve. We are presently investigating the ionic mechanisms responsible for the depolarization and bi-stable state.

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251.24 PURIFIED CALCIUM-ACTIVATED PROTEASE INCREASES GLUTAMATE BINDING TO HIPPOCAMPAL MEMBRANES. R. Siman, M. Baudry and G. Lynch, Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

Mechanisms by which the concentration of neurotransmitter receptors in the CNS are regulated are largely unknown. Recently a membrane-associated calcium-activated thiol-protease has been suggested to regulate binding of the putative transmitter glutamate to hippocampal and cortical membranes (Baudry and Lynch, 1980, PNAS 77, 2298-2302). Micromolar levels of calcium increase the density of glutamate binding sites and the increase is blocked by thiol-protease inhibitors. In order to study this mechanism we have purified and characterized calcium-activated proteases from synaptosomal plasma membranes and have tested their effects on (^{3}H)-glutamate binding.

A low ionic-strength extract prepared from crude synaptosomal plasma membranes of whole rat brain contained calcium-stimulated proteolytic activity. This was manifested as a calcium-induced increase in digestion of a casein substrate. When the extract was applied to a DEAE-cellulose column, two separate peaks of activity eluted. The fraction eluted at 100 mM NaCl (fraction I) was half-maximally activated by 40 μM calcium. In contrast, fraction II, eluted at 200 mM NaCl, had a Kd for calcium of 500 μM and showed no activation at calcium concentrations below 200 μM. Activity in both fractions was inhibited more than 80% by 50 μM leupeptin, a thiol-protease inhibitor. Fraction II protease was further purified by isoelectric precipitation and gel filtration. The purified protease adhered to a casein-Sepharose 4B affinity column in the presence of 20 mM $CaCl_2$. After elution with EGTA, the protease had a high sensitivity to calcium similar to the fraction I enzyme. The affinity-purified high-sensitivity protease altered the binding of (^{3}H)-glutamate to hippocampal membranes from 8 day old rats. Whereas 30 μM calcium caused a 15% increase in the binding of 100 nM (^{3}H)-glutamate, in the presence of added protease, calcium induced a 110% increase in binding. This enhanced calcium-stimulated binding was dependent on the dose of purified protease added, was blocked more than 90% by 50 μM leupeptin, and was not observed with affinity-purified fractions from which protease activity had been lost upon storage. These data provide a direct demonstration that a thiol-protease activated by micromolar calcium concentrations increases (^{3}H)-glutamate binding to hippocampal membranes. This high-sensitivity enzyme appears endogenous to synaptosomal plasma membranes and can be formed from a low-sensitivity protease. Supported by NIMH grant MH19793-11 and Research Scientist Award MH00358-01.

252.1 RAPID ANALYSIS OF CATECHOLAMINES, INDOLEAMINES, AND RELATED ENZYMES USING LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION, C. L. Blank, M. Bulawa,* P. Wong,* and P. Lin.* Department of Chemistry, University of Oklahoma, Norman, OK 73019.

The recent advent of liquid chromatography columns having 3 micron packing materials has greatly aided in the analysis of many compounds. Using an Ultrasphere ODS column from Altex Scientific (Beckman), we have been able to produce a separation for twelve different components (DOPA, norepinephrine, epinephrine, 5-hydroxytryptophan, DOPAC, dopamine, epinine, 5-hydroxyindoleacetic acid, homovanillic acid, 5-hydroxytryptamine, 3-methoxytyramine, and N_{ω}-methyl-5-hydroxytryptamine) with a total elution time of approx. 2 1/2 minutes.

Endogenous components are determined by homogenization of the tissue in an acetate/perchlorate buffer, centrifugation and direct injection into the LCEC. The catechols are isolated from the supernate on alumina and reinjected into the same column for quantitation. Thus, two injections onto a single LCEC setup allow quantitation of all the mentioned species.

Enzymes are analyzed either in vitro or in vivo by monitoring the products of the reactions with the same procedure. Determinations include tyrosine hydroxylase (in vivo and in vitro), tryptophan hydroxylase (in vivo and in vitro), dopa decarboxylase (in vitro), 5-hydroxytryptophan decarboxylase (in vitro), and monoamine oxidase (in vitro). The incubation parameters or pretreatments associated with these determinations have been previously mentioned elsewhere (C. L. Blank et al. in Function and Regulation of Monoamine Enzymes, eds. E. Usdin, N. Weiner, and M. Youdim, MacMillan, London, 1981, pp. 759-770).

252.2 CHRONIC RECORDING FROM CNS IN VIVO ELECTROCHEMICAL ELECTRODES: FEASIBILITY OF FOLLOWING LONG-TERM NEUROLEPTIC DRUG TREATMENT. C. W. Hughes and H. J. Pottinger*.

Recent research has demonstrated the feasibility and utility of acute recording from in vivo electrochemical electrodes designed to detect endogenous levels of CNS catecholamines. The research reported here assessed the possibilities of the acute in vivo electrochemical technique for monitoring chronic drug administration. D-amphetamine (3.0 mg/kg i.p.) administered every other day for seventeen days was followed in the anterior caudate, medial caudate, nucleus accumbens, and corpus callosum of rats.

There was no significant change in the baseline levels of electroactive species over the entire testing period for any of the brain regions, nor was there a consistent increase or decrease in the baselines over days. This finding is important for studies attempting to monitor a chronic ongoing in vivo response to drug treatment. In terms of a peak response to d-amphetamine injections, the nucleus accumbens showed a decrease for the first week followed by an increase the second week. In contrast, in the caudate there was an increase in the peak response to d-amphetamine in the first week of recording, which suggested that a "sensitization" or cellular alteration with time was being detected. No differences for baselines of peak responses to d-amphetamine were found between anterior and medial portions of the caudate, nor between left and right portions of the brain.

The results of this study support the feasibility of using the in vivo electrochemical technique for the study of brain response to chronic drug administration in animal models.

252.3 A NOVEL HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC PROCEDURE FOR SEPARATING ENDOGENOUS AMINES. P. Kontur, R. Dawson, and A. Monjan* Depts. of Envr. Hlth. Sci. and Epi., The Johns Hopkins U. Sch. of Hyg. and Public Hlth., Baltimore, MD 21205.

Studying neuronal interactions within individual brain regions demands quantification of several different neurotransmitters, their precursors and metabolites. High performance liquid chromatography with electrochemical detection is well suited for identifying and quantifying several neurochemical compounds in small brain tissue samples. A simple, rapid procedure for separating eight different amines is described.

A Du Pont 870 pump module, 860 column compartment with a 7125 Rheodyne sample injection valve (20 ul sample loop) and a BAS Biophase ODS 5 um RP-18 analytical column protected by a BAS Biophase .46 x 3 cm RP-18 Speri 5 ODS 5 um guard column comprise the LC system. A BAS LC-4A amperometric detector with a TL-5 glassy carbon electrode and HP 3390A Integrator served as detector& recorder.

A mobile phase optimized for pH and ion pairing reagent concentration was used to separate norepinephrine, epinephrine, 3,4-dihydroxyphenylacetic acid, dopamine, 5-hydroxyindole acetic acid, vanillic acid, homovanillic acid and serotonin. For optimized conditions a flow rate of 1.5 ml/min, a 45° column temperature and a potential of .72V at a sensitivity of lnA were maintained.

Experiments using 2:1 .02M citric acid-.02M phosphate buffer (.269mM EDTA) with 5.5% acetonitrile (CH_3CN) and .479mM sodium octyl sulfate (SOS) with pH varied between 2.75 and 4.00 did not result in adequate resolution of the amines. Addition of 3.962mM ammonium hydroxide (NH_4OH) improved the separation. Differential effects of pH are apparent with decreasing pH not affecting the retention of neutral amines but increasing the retention of acidic metabolites. Complete separation was achieved at a pH of 2.75.

At the optimal pH of 2.75 it was found that addition of NH_4OH resulted in a decreased retention of neutral amines and an increased retention of acid metabolites. Using .792mM NH_4OH as opposed to 3.962mM results in a decreased retention of all amines.

In a pH 2.75, citric acid-phosphate buffer, 5.5% CH_3CN mobile phase with .792mM NH_4OH, increasing the SOS concentration from .478mM to .527mM increased the retention of the amines and decreased that of the acids without improving resolution. Interestinly this increase in SOS with a mobile phase containing 3.962mM NH_4OH decreased the retention of the amines as well as that of the acids, however the overall separation is not complete.

The novel use of NH_4OH in a citric acid-phosphate buffer mobile phase allows clear separation of 8 amines. This system is currently being used to examine amine and metabolite levels in alumina extracted plasma samples as well as unextracted filtered PCA supernatants derived from homogenates of neural tissue.

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252.4

WITHDRAWN

252.5 STRUCTURAL REQUIREMENTS FOR DIFFERENTIAL INHIBITION OF ACTIVATED TYROSINE HYDROXYLASE. D.B. Bennett and C.J. Coscia. Dept. of Biochemistry, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Under conditions of cyclic AMP dependent protein phosphorylation, tyrosine hydroxylase (EC1.14.16.2) is activated. Kinetic analysis of the enzyme reveals the affinity of its cofactor, tetrahydrobiopterin, as well as the K_i of its putative feedback inhibitor, dopamine, are increased. Since this activation is considered an integral part of the sequella of impulse flow in catecholaminergic neurons and represents a mechanism to override feedback inhibition, we assessed catecholic inhibitors of tyrosine hydroxylase for the structural requirements that impart differential sensitivity to activated and inactivated enzyme. By varying cofactor and inhibitor concentrations, K_i's were generated from Dixon plots. Structural analogs of dopamine in which the amino group was fixed in a cis conformation, i.e., 6,7-dihydroxytetrahydroisoquinolines, exhibit the same K_i for activated and inactivated rat striatal tyrosine hydroxylase. However 2-amino-6,7-dihdyroxytetralin (ADTN), in which the nitrogen is extended in a fixed trans conformation of the ß-rotamer, exhibited a 4 fold increase in K_i upon assaying tyrosine hydroxylase under phosphorylation conditions. By systemetically increasing the hydrophobicity of the substituent at C-1 of 1-carboxy-6,7-dihydroxytetrahydroisoquinolines the inhibitory potency was enhanced, suggesting the presence of a hydrophobic region at the catecholic binding site. If the hydrophobic group was fixed as in the catechol estrogen, 2-hydroxy-estra-3,17ß-diol, the K_i was relatively low (2×10^{-3} M) despite the absence of an amino group and it increased 6 fold with activated tyrosine hydroxylase. These studies provide insight into the topography of the catecholic binding site on tyrosine hydroxylase and to attendant changes occurring upon activation. The results suggest that the catchol binding site includes both amino group-interacting and hydrophobic regions which are influenced by enzyme activation.
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252.6 EGTA-INDUCED ACTIVATION OF SYNAPTOSOMAL TYROSINE HYDROXYLASE: DEMONSTRATION OF INCREASED DOPA SYNTHESIS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, Dean A. Haycock* and Robert L. Patrick. Neuroscience Section, Division of Biology and Medicine, Brown University, Providence, RI 02912.

A role for calcium in regulating striatal tyrosine hydroxylase activity has been suggested based, in part, on the observation that the formation of radioactive dopamine from labeled tyrosine is increased by incubating striatal slices in a calcium-free medium (Goldstein et. al., Life Sci. (1970), 9: 919-924). It has also been suggested, however, that this increase in labeled dopamine may result from a lower rate of proteolysis in a calcium-free buffer leading to less dilution of labeled tyrosine, i.e. that the increase in labeled dopamine is due to a higher specific activity of tyrosine precursor and not to an activation of tyrosine hydroxylase (Hamon et. al., J. Neurochem. (1974), 23: 849-856). Since the use of radioactive tyrosine precludes a resolution of this issue, we have made use of high performance liquid chromatography coupled to electrochemical detection in order to measure dopa synthesis by a non-isotopic procedure in rat brain striatal synaptosomes.

This non-radioactive technique involves the measurement of dopa produced in the presence of 1.0 µM NSD-1015, an aromatic amino acid decarboxylase inhibitor. This concentration of NSD-1015 completely inhibited $^{14}CO_2$ production from L-(1-^{14}C) tyrosine, indicating complete inhibition of decarboxylase activity. Dopa formation was linear with respect to time for at least 20 minutes, and was completely prevented by the tyrosine hydroxylase inhibitor, 3-iodo-tyrosine. Synthesis assays measuring both dopa formation and the production of $^{14}CO_2$ from L-(1-^{14}C) tyrosine were run in parallel. Both assays showed a 65% synthesis stimulation by 2.0 mM dibutyryl cyclic AMP and a 70% inhibition by 2.0 µM dopamine. In the normal incubation buffer, containing 1.0 mM calcium, increasing the concentration of EGTA, a calcium chelator, to 1.0 mM revealed a statistically significant 21% synthesis stimulation in both assays. These results indicate (1) that tyrosine hydroxylation is activated by EGTA in striatal nerve-endings and that the increased synthesis observed with the radioactive assay is not an artifact of altered proteolysis, (2) that calcium may play a role in regulating striatal tyrosine hydroxylase activity in the nerve terminal in vivo and (3) that this non-isotopic technique for measuring synaptosomal tyrosine hydroxylase activity can be a useful alternative to radioisotopic techniques for clarifying the mechanisms of drug-induced alterations in catecholamine synthesis in the central nervous system.
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252.7 EFFECTS OF PHOSPHOLIPASES ON THE KINETIC PROPERTIES OF RAT STRIATAL MEMBRANE-BOUND TYROSINE HYDROXYLASE. Ronald Kuczenski, Dept. of Pharmacol., Vanderbilt Univ. School of Medicine, Nashville, TN 37232

Rat striatal tyrosine hydroxylase (EC 1.14.16.2) (TOH) can be isolated in both a soluble and a synaptic membrane-bound form. The membrane-bound enzyme, which exhibits lower Km's for both tyrosine (8 µM) and reduced pterin cofactor (110 µM) relative to the soluble enzyme (47 µM and 940 µM, respectively), can be released for the membrane fraction with mild detergent, and concomitantly its kinetic properties revert to those of the soluble enzyme.

Treatment of membrane-bound TOH with C. perfringens phospholipase C increased the Km of the enzyme for tyrosine to 30 µM and increased the V_{max} by 60% without changing the Km for cofactor. The enzyme remained bound to the membrane fraction. B. cereus phospholipase C treatment of the membrane-bound enzyme also increased the Km for tyrosine without altering the Km for cofactor. Addition of various phospholipids and/or their phosphorylated alcohol moieties did not affect the activity of control or phospholipase C-treated, washed, membrane-bound TOH. However, treated enzyme was sensitive to activation, through a two-fold increase in V_{max}, by the polyanion heparin, whereas control membrane-bound TOH is not.

In contrast, treatment of membrane-bound TOH with V. russelli phospholipase A_2 increased the Km of the enzyme for tyrosine to 45 µM, increased the V_{max} by 60%, and also increased the Km for cofactor to 500 µM. Again, the enzyme remained bound to the membrane fraction. The kinetic properties of phospholipase-treated detergent solubilized TOH were identical to control solubilized TOH.

Soluble rat striatal TOH, like the rabbit adrenal enzyme (Lloyd, J. Biol. Chem. 254, 7649, 1979) could be activated, through a decrease in Km for cofactor, by certain phospholipids. However, under no conditions did incubation of the soluble TOH with phospholipids mimic the effects of membrane-binding on the Km for substrate.

Rat striatal TOH appears to interact with synaptic membrane components to produce at least two separable consequences for the kinetic properties of the enzyme. Membrane phospholipids may directly mediate these changes in kinetic properties. Alternatively, since soluble TOH in the presence of added phospholipids or their components fails to mimic all of the kinetic properties of the intact membrane-bound enzyme, membrane phospholipids may play an indirect role by maintaining a specific membrane conformation through which the enzyme can interact with other membrane components. (Supported by Grant #BNS 80-22441 from NSF).

252.8 EVIDENCE FOR HOMOLOGOUS PROTEIN DOMAINS AMONG CATECHOLAMINE SYNTHESIZING ENZYMES REVEALED BY FINGER PRINTING ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. M.E. ROSS, C.Y. LAI*, D.J. REIS AND T.H. JOH. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021 and Roche Institute of Molecular Biology, Nutley, NJ 07110.

We have proposed that the catecholamine synthesizing enzymes, tyrosine hydroxylase (TH), dopamine B-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) probably share common gene coding sequence(s) which are reflected in the primary structures of these three enzymes (Joh et al., Fed. Proc. 41:1705, 1982). Evidence includes the fact that limited proteolytic cleavage of each enzyme yielded large peptides with similar molecular weights, as resolved on SDS PAGE, and these peptides also had equivalent amino acid compositions.

In the present study small to medium sized peptides generated by extensive proteolysis were compared by finger print analysis using high performance liquid chromatography (HPLC). TH, DBH and PNMT were purified to homogeneity from bovine adrenal medulla. Purified enzymes were reduced and carboxymethylated with 2-(^{14}C)-iodoacetic acid and digested for 16 h at 25°C with trypsin which had been treated with tosyl phenylcarboxymethyl ketone (TPCK) to inactivate exogeneous chymotrypsin activity. Equimolar amounts of each digest were the subjected to chromatography under exactly the same conditions on reversed phase HPLC and resolved peptides were counted to detect the presence of ^{14}C carboxymethyl cysteine. Peptides with similar retention times were hydrolyzed for amino acid analysis.

Comparison of HPLC data revealed a striking 66% overlap of peptides with similar retention times between TH and DBH, and 25 to 38% overlap between PNMT and TH or DBH, respectively. Amino acid content of these peptides showed a 35-40% homology between TH and DBH, respectively, and at least a 10-20% homology between PNMT and TH or DBH respectively.

These results indicate that all three enzymes have protein domains with similar primary structure. This is consistent with our hypothesis that TH, DBH and PNMT share common gene coding sequence(s), and that these enzymes have evolved from a common ancestral precursor.

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252.9 TIME COURSE STUDY OF CHANGES IN THE ACTIVITY OF THE CATECHOLAMINE SYNTHESIZING ENZYMES OF THE RAT MEDULLA OBLONGATA AFTER INTRAVENTRICULAR INJECTION OF 6-HYDROXYDOPAMINE. R. FETY* and B. RENAUD. Laboratoire de Neuropharmacologie and ERA CNRS 894, Faculté de Pharmacie, Université Claude Bernard, Lyon, France.

Recent data have shown that the central noradrenergic (NA) cell bodies of the locus coeruleus (LC) respond to an intraventricular injection of 6-hydroxydopamine (6 ODHA) by an increase in tyrosine hydroxylase (TH) activity 5 to 21 days after the injection (ACHESON et al., Science, 1980, 207, 537-539). In this study, we sought to determine if injection of 6 OHDA into the fourth ventricule induces such a compensatory alteration within two other groups of central NA neurons, namely the A1 and A2 neuronal groups of the rat medulla oblongata. To follow the responses of NA neurons at various times after 6 OHDA injection, we used as markers the "in vitro" activities of the two catecholamine synthesizing enzymes TH and dopamine-β-hydroxylase (DBH). Although 6 OHDA does not seem to alter the adrenergic (A) neurons, the adrenaline synthesizing enzyme phenylethanolamine-N-methyltransferase (PNMT) was used as specific marker of the A neurons (C1 and C2 groups) and its "in vitro" activity was determined simultaneously with TH and DBH activities in the LC, in the A1-C1 and in the A2-C2 areas. In order to measure the changes occurring not only in the cell bodies but also in their corresponding terminals, the enzymatic activities were also determined in the tractus intermediolateralis (TIML) of the spinal cord, an area rich in NA and A terminals originating mainly in the LC, A1-C1 and/or A2-C2 brain areas.

The TH activity within the cell bodies was significantly increased 2 days after the 6 OHDA injection with a maximum at 5 days (LC : +109%, $P < 0.001$; A1-C1 : +40%, $P < 0.01$; A2-C2 : +24% $P < 0.01$). Twelve days after injection, there was no increase in TH activity and at day 21 a significant decrease was present (LC: -36%, $P < 0.001$; A1-C1 : -18%, $P < 0.05$; A2-C2 : -26%, $P < 0.001$). Conversely, the DBH activity was never increased after 6 OHDA injection and exhibited a maximal decrease at day 21 (LC : -41%, $P < 0.001$; A1-C1 : -33%, $P < 0.001$; A2-C2 : -40%, $P < 0.001$). The PNMT activity was never modified after the 6 OHDA injection.

The TH activity within the terminals of the TIML was significantly decreased at each point with a maximal decrease at day 5 (-47%, $P < 0.001$). The DBH activity exhibited a parallel but more pronounced decrease (-82%, $P < 0.001$) at day 12. The PNMT was unchanged in the TIML after injection.

These data demonstrate that the NA neurons of the rat medulla oblongata and of the LC exhibit a similar pattern of response to the neurotoxin 6 OHDA. Conversely, the absence of change in the PNMT activity confirm the idea of a resistance of the A neurons to 6 OHDA or question the validity of the PNMT as a A marker.

252.10 PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN CULTURED PC12 CELLS. K. Lee[1*], E. Sabban[1], M. Goldstein[1], P.J. Seeley[2*] and L.A. Greene[2*] (SPON: R. Margolis). Depts. of Psychiatry[1] and Pharmacology[2], New York Univ. Med. Ctr., New York 10016.

Tyrosine hydroxylase (TH) can be phosphorylated by a c-AMP-dependent and/or -independent protein kinase. We have now investigated the phosphorylation of TH in PC12 cells under various experimental conditions. Monolayers of rat pheochromocytoma PC12 cells were labelled with (^{32}P) orthophosphate for 1-2 hours in a HEPES-buffered Ringer's medium supplemented with various additives (see below). At the end of the incubation the cells were washed and lysed, and aliquots were taken to determine the radioactivity incorporated into macromolecules (TCA precipitate). The enzyme was immunoprecipitated with specific rat anti-TH antibodies (K.A. Markey et al., Mol. Pharmacol. 17:79, 1980). The immunoprecipitated TH was subjected to SDS-PAGE electrophoresis and the phosphorylated enzyme was detected by autoradiography. A single (^{32}P) labelled band was detected which had the same electrophoretic mobility as purified TH from PC12 cells. The extent of phosphorylation was measured in samples containing equal amounts of TCA precipitable radioactivity. The phosphorylation of TH was significantly increased in the presence of high K^+ (40 mM). This effect appears to require the presence of extracellular Ca^{++} and to be mimicked by the Ca^{++} ionophore A23187. The addition of dB-cAMP (1 mM) or of the phosphodiesterase inhibitor, IBMX (0.1 mM) also resulted in an increased phosphorylation of TH. The phosphorylation of TH was increased to a greater extent in the presence of high K^+ than in the presence of dB-cAMP. In presence of high K^+, the addition of dB-cAMP or of IBMX resulted in an additional increase of the phosphorylation. In agreement with a previous report (S. Halegoua and J. Patrick, Cell, 22:571, 1980) exposure of cells to NGF (50 ng/ml) resulted in an increase of TH phosphorylation. The finding that TH phosphorylation is enhanced by elevated extracellular K^+ may be of physiological importance. One may postulate that the increased catecholamine biosynthesis evoked by depolarizing agents, such as K^+, involves phosphorylation of TH. We are now further investigating the mechanisms involved in the K^+- and NGF-elicited enhanced phosphorylation of TH. Supported by NINDS 06801 and NIMH 02717 (M.G.) and NS 16036 and March of Dimes Birth Defects Foundation (L.A.G.).

252.11 ACTIVATION OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE BY PHOSPHATE. D.H. Park, P.R. Kennedy*, D.J. Reis and T.H. Joh, Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Phenylethanolamine N-methyltransferase (PNMT) catalyzes the conversion of norepinephrine to epinephrine. In bovine adrenal medulla it exists as charge isozymes. The charge difference is also observed between species; PNMT from rat adrenal gland (rat form) migrates faster than that from bovine adrenal medulla (bovine form) on PAGE, although both forms of PNMT are similar in their primary structures.

We sought to investigate etiology of the charge difference between two forms. When 100 mM potassium phosphate (KP) was added to the enzyme assay mixture (using Tris-Cl as the buffer base), activity of the purified bovine form increased maximally 330% from 52 nmole/mg protein/hr (units) to 226 units, while the rat form increased 40% from 80 to 114 units. In a crude PNMT preparation, activity increases reached 560% for the bovine form (8.6 to 57 units) and 60% for the rat form (10 to 16 units). Other negatively charged ions, e.g. sulfate and chloride, had less effect on the activity of the bovine form (sulfate increases 100%) and no effect on the rat form. Positive ions, such as Tris, had no effect. Since charge differences in the bovine isozymes are due to the presence of carbohydrate, we sought to determine whether elimination of a sialic acid moiety from the enzyme by neuraminidase alters PNMT activity and/or the phosphate effect. Pretreatment with PNMT with neuraminidase (0.5 U) caused no significant effect on PNMT activity of either form. Addition of phosphate (100 mM) to the neuraminidase-treated mixture produced less activation of the bovine form (control = 210 and treated = 106 units) and had no effect on the rat form.

The results indicate that: (1) bovine PNMT differs from rat in its mechanism of activation by phosphate, and (2) phosphate activation of PNMT is probably due to association of the ion with a sialic acid moiety. Thus the observed difference between bovine and rat forms is probably due to the sialic acid content of either enzyme, reflecting species differences in the posttranslational modification of PNMT as previously proposed (Park et al. J. Neurochem. 38: 410, 1982).

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252.12 CHARACTERIZATION OF BOVINE ADRENOMEDULLARY DOPA-DECARBOXYLASE. V.R. Albert*, D.J. Reis and T.H. Joh. (SPON: M. P. Meeley). Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

DOPA decarboxylase (DDC) is the enzyme responsible for the conversion of L-DOPA to dopamine and 5-OH tryptophan to serotonin. The enzyme also catalyzes the decarboxylation of several aromatic amino acids and has been found in both neuronal and non-neuronal tissues. In the present study, we have sought to characterize the enzyme using biochemical, immunochemical and molecular biological techniques.

DDC was purified from bovine adrenal medulla by 30-45% Ammonium Sulfate fractionation, DEAE-Cellulose chromatography, and gel filtration on Sephadex G-200. The enzyme thus purified was subjected to PAGE to ascertain homogeneity. Enzymatically active bands eluted from the gels produced a single band when subjected again to PAGE and were judged to be 99% pure by SDS-PAGE. The molecular weight was determined to be 56,000. The K_m for L-DOPA and Tyrosine were 1.4×10^{-5} M and 7.5×10^{-3} M respectively, values similar to those obtained for kidney DDC. Antibodies were raised in two rabbits by injecting homogenized polyacrylamide gel slices containing enzymatically active protein. The antibodies were determined to be specific for DDC by double immunodiffusion and immunoelectrophoresis. Both antibodies specifically localized catecholamine and serotonin containing neurons in the rat by immunohistochemistry. Immunodiffusion and immunochemical titration experiments revealed no differences in cross reactivity of the antibodies with enzyme from rat or bovine liver, kidney, brain or adrenal medulla, confirming the results of Christenson et al. (PNAS 69:343, 1972), indicating that the enzyme is essentially the same in all tissues.

Immunoprecipitation of bovine adrenal poly(A)mRNA translation products suggests that DDC is synthesized in the adrenal medulla as a 78,000 MW precursor. Total RNA was purified from bovine adrenal medulla and poly(A)mRNA was isolated by oligo-dT Cellulose column chromatograaphy. Poly(A)mRNA was translated in a reticulocyte lysate translation system and protein products were immunoprecipitated with both DDC antibodies in the presence of protease inhibitors (PMSF, 5×10^{-4} M and trypsin inhibitor, 0.01 mg/ml). The major precipitated protein had a molecular weight of 78,000 daltons on SDS-PAGE. In the absence of protease inhibitors, both antibodies precipitated a 56,000 MW protein. The results thus suggest that the precursor of DDC is a 78,000 MW protein and that proteolytic degradation during post translational modification yields the 56,000 MW form of DDC.

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252.13 6-METHYLTETRAHYDROBIOPTERIN PREVENTS THE METHAMPHETAMINE-INDUCED DEPRESSION OF TYROSINE HYDROXYLASE AND TRYPTOPHAN HYDROXYLASE IN VARIOUS BRAIN REGIONS. T.M. Cook* and J.W. Gibb (SPON: L. Jarcho). Dept. Biochem. Pharmacol. and Toxicol., University of Utah, Salt Lake City, Utah 84112.

The rate-limiting step in the biosynthesis of catecholamines is the conversion of tyrosine to L-dopa by tyrosine hydroxylase (TH). TH requires both molecular oxygen and the reduced form of the hydroxylase cofactor, tetrahydrobiopterin (BH_4). The initial and rate-limiting reaction in the biosynthesis of 5-hydroxytryptamine (5-HT) in central serotonergic neurons is catalyzed by tryptophan hydroxylase (TPH). TPH activity also requires BH_4. Both chronic and acute injections of methamphetamine (METH) significantly decrease TH and TPH activity in selected rat brain regions (Bakhit et al., Eur. J. Pharmacol., 76:229, 1981; Morgan et al., Neuropharmacol., 19:989, 1980). Peripheral administration of BH_4 and 6-methyltetrahydrobiopterin ($6MPH_4$) increases brain levels of biopterin by 2 and 10 times respectively (Kapatos et al., Science 212:955, 1981). The biosynthetic pathway and BH_4 are localized in dopaminergic nerve terminals in the striatum (Levine et al., Science 214:919, 1981). Studies of the regional and subcellular distribution of the pteridine cofactor have suggested that a significant portion of the total reduced pterins in the brain are highly correlated with the catecholamine neurons rather than the serotonin neurons (Bullard et al., J. Pharm. Exp. Ther., 206:4, 1978; Mandell et al., in Depressive Disorders, 13th Symposium Med. Hoescht, 179, 1977). Intraventricular infusion of BH_4 increases striatal TH activity; however intravenous injection of BH_4 caused only a slight increase in TH activity (Kettler et al., Nature 249:476, 1974). Striatal BH_4 levels are decreased to 70% of controls following administration of d-amphetamine (Mandell et al., J. Pharm. Exp. Ther., 213:569, 1980).

The present study examined the effects of simultaneous injections of $6MPH_4$ and METH on TH and TPH activity. Rats were injected with METH (10 mg/kg, s.c.) and $6MPH_4$ (1 mM or 2 mM, i.p.) and sacrificed after 3 hrs. Rats were killed by decapitation and the following brain regions were dissected: neostriatum (NS) hypothalamus (HY), hippocampus (H) and cerebral cortex (CC). TH activity was measured by the method of Nagatsu et al., (Anal. Biochem., 9:122, 1964). TPH activity was measured by a modified $^{14}CO_2$-trapping method (Hotchkiss et al., Life Sci., 25:1373, 1979). TH activity in METH treated animals was decreased in the striatum to 68% of control values. The simultaneous injection of $6MPH_4$ prevented the METH-induced depression. TH activity in the HY was reduced to 86% of controls; again, the presence of $6MPH_4$ prevented the decrease. TPH activity was also markedly reduced following the METH injection: H 69%, HY 68%, CC 53% and NS 55%. The $6MPH_4$ injection appeared to prevent the METH-induced decrease in TPH activity. (Supported by USPHS grant DA 00869).

252.14 MEASUREMENT OF 1-(4-HYDROXY-3-METHOXY)ETHANE-1,2-DIOL (MOPEG) IN MOUSE BRAIN AND CAT CSF BY HPLC WITH ELECTROCHEMICAL DETECTION; EFFECT OF YOHIMBINE AND MIANSERIN. Richard A. Ferrari*, Mary J. Connell*, Robert G. Ferraino* and Dean R. Haubrich. Dept. of Pharmacology, Neuropsychopharmacology Section, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.

MOPEG is a major metabolite of norepinephrine in the central nervous system. We have developed an improved and reproducible method for quantitating MOPEG and have used this procedure to evaluate drugs.

Mouse brain and cat CSF were employed because >98% of the MOPEG is present in the free form. No hydrolysis or preliminary separations were necessary. Acid hydrolysis was not reproducible in our hands and enzymatic hydrolysis of conjugates was incomplete, necessitating large correction factors. The present method which has been used by us for one year, gives 90% recovery of MOPEG in brain well separated from other peaks, is reproducible and is sensitive to 10 pg at a signal to noise ratio of 6.

Mouse brains were homogenized in 40% ethanol containing EDTA and ascorbate, centrifuged and the NaCl-saturated supernatant was extracted twice with ethyl acetate. The organic phase was evaporated with N_2, taken up in 200 µl of 0.1M HCOOH containing EDTA and ascorbate and injected onto the HPLC after centrifuge filtration. Cat CSF was injected into the HPLC without extraction. The columns used were a 3 cm, 5µm C-18 guard column and a 25 cm, 5µm C-18 analytical column. The mobil phase was 0.1M sodium acetate containing 10^{-4}M EDTA and 5% methanol at pH 5. A Bioanalytical Systems glassy carbon electrochemical detector was used without need for frequent resurfacing.

The concentration of MOPEG was 45 ± 2.5 ng/g in mouse brain (N=47) and 9.7 ± 0.3 ng/ml (N=26) in cat CSF (cisterna; chloral hydrate anesthesia). In mice treated subcutaneously with yohimbine (10 µmole/kg) or mianserin (40 µmole/kg) a maximal increase in MOPEG occurred at 1 hr, and returned to normal within 8 hrs after treatment. Treatment of cats with yohimbine s.c. caused a prolonged increase in CSF levels of MOPEG which lasted for more than 3 hrs. These results indicate that MOPEG measured by HPLC/EC reflects changes in norepinephrine release by adrenergic antagonists.

252.15 EFFECTS OF HALPERIDOL, PIMOZIDE, CLOZAPINE AND SULPIRIDE ON THE LONG-TERM EFFECTS OF AMPHETAMINE ON STRIATAL DOPAMINE NEURONS IN IPRINDOLE-TREATED RATS. L.R. Steranka. Northwest Center for Medical Education, Indiana University School of Medicine, Gary, IN 46408.

The decreases in striatal dopamine (DA), 3,4-dihydroxphenylacetic acid (DOPAC) and homovanillic acid (HVA) at 1 week after the administration of a single dose of (+)-amphetamine sulfate (9.2 mg/kg) to rats treated with iprindole hydrochloride (10 mg/kg) were prevented by haloperidol (1 mg/kg) and pimozide (2 mg/kg). Clozapine (40 mg/kg) failed to attenuate the decreases in striatal DA, DOPAC and HVA at 1 week after amphetamine at a dose that prevented the rotational behavior induced by the administration of amphetamine sulfate to iprindole-treated rats with unilateral aspiration lesions of the striatum. In contrast to clozapine, sulpiride (32 mg/kg) prevented the amphetamine-induced decrease in striatal DA 1 week after drug administration at a dose that did not alter the effect of the drug on rotational behavior. None of these neuroleptic drugs altered the concentration of amphetamine in the brains of iprindole-treated rats at 8 hours after drug administration, which corresponds to approximately 2 half-lives of amphetamine in brain after iprindole treatment. Taken together, these results suggest that (1) a critical step in the sequence of events leading to the production of the apparent neurotoxic effect of amphetamine on striatal DA neurons is sensitive to inhibition by a variety of neuroleptic drugs; (2) the blockade of postsynaptic DA receptors per se does not attenuate the ability of amphetamine to produce neurotoxic effects on striatal DA neurons; and (3) the ability of sulpiride to prevent the persistent decrease in striatal DA is related to some action of drug other than the blockade of postsynaptic DA receptors in the striatum.

This study was supported in part by the Lake County Medical Center Development Agency (IN) and by a Research Starter Grant from the Pharmaceutical Manufacturers Association Foundation, Inc.

252.16 DIFFERENTIAL EFFECTS OF SEVERAL CENTRAL NERVOUS SYSTEM STIMULANTS ON THE EFFLUX OF DIHYDROXYPHENYLACETIC ACID (DOPAC) INTO THE VENTRICULAR PERFUSATE OF UNANESTHETIZED RATS AND ON THE RATE OF DOPAMINE SYNTHESIS IN RAT STRIATUM. J.A. Nielsen* and K.E. Moore (SPON: S.R. Heisey). Dept. of Pharmacology/Toxicology, Michigan State Univ., East Lansing, MI 48824.

Central nervous system stimulants are believed to interact with dopamine (DA) neurons in the brain. The present study evaluated the effects of these drugs on the efflux of DOPAC into lateral ventricular perfusates of unanesthetized rats, and compared these effects with stimulant-induced changes in the rate of DA synthesis in the striatum.

Male rats were implanted with permanent push-pull cannulas such that the tips were in the right lateral ventricles (Pharm. Biochem. Behav. 16: 131, 1982). After recovery from surgery the cerebroventricular system of each rat was perfused with artificial cerebrospinal fluid at a rate of 20 µl/min, and perfusate samples were collected in a high performance liquid chromatography (HPLC) mobile phase (0.1 M citrate-phosphate buffer, pH 3.0, containing 8% methanol, 0.024% sodium octyl sulfate and 0.1 mM disodium ethylenediamine tetraacetate) every 15 min for 45 min before and up to 135 min after the subcutaneous injection of a stimulant drug. The concentration of DOPAC was analyzed by HPLC using a C_{18} µBondapak column coupled to an electrochemical detector. The in vivo rate of DA synthesis was estimated in different male rats by radioenzymatically measuring the rate of accumulation of DOPA in the striatum 30 min after the administration of an inhibitor of aromatic L-amino acid decarboxylase (NSD 1015; 100 mg/kg, i.p.).

d-Amphetamine produced a dose dependent change in the efflux of DOPAC into ventricular perfusate and DOPA accumulation in the striatum. DOPAC efflux and DOPA accumulation were increased after the administration of a low dose (1 mg/kg), while a higher dose (5 mg/kg) had the opposite effect. On the other hand, methylphenidate (4-8 mg/kg) administration caused a dose-related decrease in both the efflux of DOPAC and the rate of DA synthesis in the striatum; cocaine (10-20 mg/kg) had similar effects. Finally, β-phenethylamine increased the perfusate content of DOPAC. The effects of these drugs on the efflux of DOPAC and the rate of DA synthesis is complicated by their multiple actions on the dynamics of DA at the terminals of nigrostriatal neurons. These actions include facilitation of DA release, depletion of selected intraneuronal pools of DA, inhibition of neuronal reuptake of DA, and activation of neuronal- and autoreceptor-mediated feedback mechanisms. (Supported by USPHS grant NS15911.)

252.17 DRUG-INDUCED RELEASE OF DOPAMINE IN AGGREGATE COCULTURES OF MESENCEPHALIC TEGMENTUM AND CORPUS STRIATUM. Ismail Shalaby, Connie Kotake*, Philip Hoffmann* and Alfred Heller. Dept. of Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, Il., 60637.

Dopamine (DA) neurons from the rostral mesencephalic tegmentum (RMT) of embryonic mouse brain were dissociated and allowed to aggregate in vitro with dissociated cells from the corpus striatum (CS). Fluorescence histochemical observations indicate that DA cells of RMT form a dense axonal plexus only in the presence of target cells of striatum (Hemmendinger et al., PNAS, 78:1264, 1981). When cultured over a 21 day period such coaggregates exhibit a developmental increase in DA levels and uptake, an increase in tyrosine hydroxylase activity, and enhanced reserpine and 6-OHDA-induced DA depletions (Kotake et al., J. Neuroscience, In press). The present study assessed the capacity of these cultured DA neurons to release DA under various conditions. Coaggregates of RMT-CS maintained for 17-21 days in culture were exposed to 5.6 X 10^{-6} M ^{3}H-DA for 30 min. The tissue was then superfused at a rate of 100µl/min with a Krebs-Ringer buffer containing 100µM pargyline. Fractions were collected every 2 min and ^{3}H was counted. Results are expressed as % of tissue stores of DA released/2 min at the peak of the response. During basal efflux, DA constituted 37% and 3-methoxytyramine (3-MT) 38% of the total ^{3}H content per fraction. During stimulated release DA was 66% and 3-MT 21% of the total. After stable basal efflux of ^{3}H-DA was reached (0.8-1.0% of tissue stores), coaggregates were exposed to buffer containing 50 or 70mM potassium for 8 min. 50mM K^+ induced a peak release of 2.25 ± 0.21% (S.E.M.) and 70mM K^+ induced a release of 5.25 ± 0.79% of tissue ^{3}H-DA. The K^+-induced release was calcium dependent. When basal efflux had stabilized again, the same coaggregates were exposed to various concentrations of d-amphetamine or substance P (SP) for 12 min. d-Amphetamine induced a dose-dependent release of ^{3}H-DA. 100µM concentrations of d-amphetamine induced a peak release of 8.91 ± 0.96%; a 10µM solution released 4.44 ± 1.21%, and a 1µM solution induced a peak release of 1.81 ± 0.47% of tissue stores of ^{3}H-DA. Coaggregates exposed to 100µM SP released ^{3}H-DA to a peak of 2.35 ± 0.18% of tissue ^{3}H-DA stores. 10 and 1µM solutions of SP induced peak releases of 1.21 ± 0.27% and 1.02 ± 0.24%, respectively.

Taken together with our previous results, these experiments demonstrate that dissociated mesencephalic embryonic DA neurons when allowed to aggregate with striatal target cells in culture, behave in a manner qualitatively indistinguishable from nigrostriatal DA neurons in vivo. These aggregate cultures, amenable to manipulative studies not possible in vivo, should provide new information on the neurochemical regulation of DA neurons in the intact brain. (Supported by: US PHS MH 10717 and GM 07151-07)

252.18 EXCHANGE DIFFUSION, THE CYTOPLASMIC DOPAMINE POOL, AND AMPHETAMINE ACTIVATION OF SYNAPTOSOMAL DOPAMINE SYNTHESIS. Charles Connor and Ronald Kuczenski, (SPON: E. Sanders-Bush), Dept. Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232

Dopamine (DA) synthesis in rat striatal synaptosomes may be partially regulated by the influence of an inhibitory cytoplasmic pool of DA on the rate-limiting enzyme, tyrosine hydroxylase. Amphetamine (AMPH), which is thought to release DA from the cytoplasmic pool via an accelerative exchange diffusion process involving the DA uptake carrier molecule, caused approximately a 40% activation of synthesis at doses between 10^{-6} and 10^{-5} M. This phenomenon showed only a minor Ca^{++} dependency. The activation could be abolished by the DA uptake carrier blocker nomifensine (NMF) (10^{-5} M). Conditions which reverse the Na^+ gradient across the synaptosomal membrane promote release of cytoplasmic DA, perhaps because of the dependency of the DA carrier molecule function on Na^+ cotransport. Lack of K^+ in the incubation medium, which inhibits the Na^+-K^+-ATPase pump, allowing accumulation of Na^+ inside the synaptosome, activated synthesis approximately 20%. AMPH activation of synthesis in the absence of K^+ was enhanced to 95% (relative to controls with K^+), presumably because the change in Na^+ gradient favored the outward transport of DA induced by AMPH. Both of these effects were prevented by NMF. Replacement of Na^+ in the medium by choline or sucrose activated synthesis by 70%. The addition of AMPH had no further effect in this case, possibly because Na^+ was needed for the inward transport of AMPH necessary for exchange diffusion to occur. NMF did not prevent the no-Na^+ activation, perhaps because Na^+ is necessary for binding of NMF to the carrier molecule.

AMPH activation was also influenced by manipulations which alter the size of the cytoplasmic DA pool. Preincubation of the synaptosomes at 37° C in modified Krebs Ringer Phosphate medium abolished AMPH activation by increasing baseline synthesis until it matched synthesis in the presence of AMPH. The addition of 10^{-5} M clorgyline prevented the increase in baseline synthesis, permitting AMPH activation. Reserpine (2 x 10^{-7} M) also reversed the effects of 37° C preincubation and permitted AMPH activation. NMF did not affect the increase in synthesis caused by 37° C preincubation. These results suggest that during 37°C preincubation, the cytoplasmic pool of DA is degraded by monoamine oxidase or depleted by vesicular uptake, thus eliminating the possibility for AMPH activation.

These data are consistent with the theory that AMPH activates synaptosomal DA synthesis by the release via accelerative exchange diffusion of an inhibitory cytoplasmic DA pool. (Supported by Grant #DA02676)

252.19 PHARMACOLOGICAL EFFECTS OF SULFONIUM ANALOGS OF DOPAMINE AT DOPAMINERGIC SYNAPSES IN STRIATUM. B.S. Turowski*, A.K. Kuruvilla*, M. Szkrybalo*, K. Anderson*, D.D. Miller* and N.J. Uretsky. Coll. of Pharmacy, Ohio State Univ., Columbus, OH 43210.

We have synthesized analogs of dopamine (DA) in which the nitrogen atom is replaced by a sulfonium (S^+) group. The purpose of these studies is to determine the effects of these analogs on pre- and postsynaptic DA mechanisms. The S^+ analogs studied were [2-(3,4-dihydroxyphenyl)ethyl]dimethyl sulfonium iodide (Analog I), [2-(3,4-dihydroxyphenyl)ethyl]dimethyl sulfonium iodide (Analog II), and [2-(3,4-dihydroxyphenyl)-2-oxoethyl]-pentamethylene sulfonium iodide (Analog III).

In order to evaluate the potency of the S^+ analogs as DA agonists, we have examined their ability to inhibit K^+-induced release of ^{3}H-acetylcholine (Ach) from striatal slices. The slices were preincubated with ^{3}H-choline (0.1 µM) and then superfused. ^{3}H-Ach release was induced by exposure to K^+ (12.5 mM) and the effects of drugs determined. All of the S^+ analogs inhibited the release of ^{3}H-Ach with a maximum reduction similar to that produced by DA. The IC50 of analogs I, II, and III were 8, 35, and 45 µM, respectively. The IC50 for apomorphine and DA were 0.013 µM and 1.3 µM. The inhibition of ^{3}H-Ach release by analog I and apomorphine was blocked by fluphenazine (1 µM). These results further support the hypothesis that the S^+ analogs exert postsynaptic DA agonist activity.

To determine whether these analogs act directly on DA receptors, we have examined their ability to inhibit the high affinity binding of ^{3}H-spiperone (0.25 nM) and ^{3}H-DA (2 nM) in a striatal membrane preparation. Analogs I and III, but not II, inhibited ^{3}H-spiperone binding with very low potency. However, the S^+ analogs were several orders of magnitude more potent in inhibiting ^{3}H-DA binding. The inhibitory potency of both ligands was less than that of DA. These results indicate that the S^+ analogs bind to DA receptors with a pattern consistent with a DA agonist action.

To determine whether presynaptic effects of the S^+ analogs participate in their agonist action, we have studied the ability of analog I to block uptake and/or to cause the release of ^{3}H-DA from striatal slices. Analog I inhibited ^{3}H-DA uptake by approximately 20 and 56% at 10 and 100 µM, respectively. In addition, analog I markedly stimulated the spontaneous release of ^{3}H-DA at 10 and 100 µM.

These results show that the S^+ analogs of DA can act on both presynaptic DA nerve terminals and postsynaptic DA receptors. Thus, compounds produced by the replacement of the nitrogen atom of DA with a S^+ group can release DA, inhibit DA uptake, and activate DA receptors. Supported by NS 17907.

252.20 DIFFERENTIAL DENSITY OF α-ADRENERGIC RECEPTOR SITES IN SPINAL CORD. N. Schechter, E. Edwards*, J.B. Cabot, and N. Bogan*, (SPON: J. Wilson). Depts. of Psychiatry, Neurobiology & Behavior and Long Island Research Institute, SUNY at Stony Brook, Stony Brook, NY 11794.

Vertebrate spinal cord contains catecholamine(CA) terminals within the dorsal horn, ventral horn and neuropil surrounding sympathetic preganglionic cells(SPN). The physiological role of such input is a matter of current debate, and even less is known about the possible classes of receptors which might be mediating the effects of spinally released CA. With respect to the putative neurotransmitter role of CA within the SPN neuropil, recent iontophoresis experiments in the pigeon (Guyenet, P.G. and Cabot, J.B., J. Neurosci., 1:908, 1981) suggest that endogenously released CA inhibits the maintained discharge of SPN. This study also proposed that these effects might be mediated by α_2 receptors not located presynaptically on CA terminals. The present series of biochemical studies represent a continuation of these efforts and are designed to test the hypothesis that there are more α-adrenergic receptors in thoracic spinal cord than in cervical spinal cord due to the unique presence in the former of the sympathetic preganglionic cell column.

Adrenergic receptor binding activity was determined according to the method of U'Prichard et al.(Mol. Pharmacol., 16:47, 1979). Pigeons(Columba livia) were decapitated and spinal cord segments C2-10 and T1-5 were removed and homogenized separately in 20 vols of cold 50 mM Tris(pH 7.7). Incubations contained 1.5-2.0 mg of protein of a resuspended particulate fraction (40,000 x g). ^{3}H-para-aminoclonidine (^{3}H-PAC) or ^{3}H-clonidine (^{3}H-CLN) was used to determine the concentrations of α-adrenergic receptors. Non-specific binding was determined by parallel incubations containing 1 µM cold clonidine.

Saturation analysis indicates that the Kd for CLN in thoracic and cervical spinal cord is 15.4 and 14.7 nM, respectively; the Kd for PAC is 4.0 and 4.9 nM, respectively. There are marked, highly significant differences (ANOVA, $p<0.01$) in the number of binding sites (B_{max}) in thoracic and cervical spinal cord when ^{3}H-CLN or ^{3}H-PAC are employed as probes. Experiments with both ligands indicate that the density of receptor sites is 31-49% greater in thoracic than in cervical spinal cord. Specifically, the concentration of sites with ^{3}H-CLN is 51.5 fmol/mg protein in thoracic cord and 34.4 fmol/mg protein in cervical cord; with ^{3}H-PAC the density of receptor sites is 64.0 and 49.0 fmol/mg protein for thoracic and cervical spinal cord, respectively. Preliminary studies suggest that a significant proportion of the observed differences are due to an increase in the number of high affinity sites. (Supported by HLBI grant HL24103).

252.21 CELLULAR SITES FOR POST-SYNAPTIC DOPAMINE METABOLISM IN RAT STRIATAL SLICES BY TYPE A AND B MONOAMINE OXIDASE. A.J. Azzaro, D.D. Schoepp*, G. Carter*, and J. King*. Departments of Neurology, Pharmacology/Toxicology and Behavioral Medicine, West Virginia University Medical Center, Morgantown, WV 26506.

Intrastriatal injections of kainic acid were utilized to investigate the cellular localization and function of non-dopaminergic type A and B monoamine oxidase (MAO) in rat striatum. Degenerative changes following kainic acid were characterized using biochemical markers for different cell types. The type A and B forms of MAO were also measured in an attempt to determine the form(s) present within populations of degenerating and proliferating cells. Further experiments were designed to determine if neuronal degeneration and gliosis might lead to altered post-synaptic dopamine (DA) metabolism.

At two days post-injection, maximal decreases in the activities of choline acetyltransferase (49% of control) and glutamic acid decarboxylase (55% of control) were observed, and found to be associated with decreases in type A and B MAO activity to 91% and 77% of control, respectively. An increase in the activity of the glial enzyme glutamine synthetase was also observed at 2 days (126% of control); however, changes in this enzyme were not maximal until at least 8 days (167% of control). During this latter time period, a return to normal in type B MAO activity was observed. These results suggest that gliosis per se is associated with a selective increase in striatal type B MAO activity. Furthermore, a subpopulation of kainic acid-sensitive neurons appear to contain both the type A and B MAO.

When the metabolism of ^{3}H-DA (10^{-7}M) was examined in kainic acid-lesioned rat striatal slices (8 days post-injection), an elevation in ^{3}H-homovanillic acid (HVA) and ^{3}H-dihydroxyphenylacetic acid (DOPAC) formation was observed. The elevation in ^{3}H-DOPAC, but not ^{3}H-HVA, was inhibited by the DA-neuronal uptake inhibitor, nomifensine. This is consistent with early findings suggesting the HVA is formed exclusively within a site(s) outside the DA neuron. Experiments conducted in the presence of the selective MAO-inhibitors, clorgyline and/or deprenyl demonstrated that while ^{3}H-HVA formation was enhanced in lesioned tissue, the relative roles of the type A and B enzymes were not altered. This metabolism, as in control tissue, primarily involved the type A MAO; however, a minor role for the type B MAO (about 10% of DA deamination) could be demonstrated, in the absence of the type A enzyme. When a higher concentration of ^{3}H-DA was used (10^{-5}M), a greater type B MAO activity component was observed; representing about 30% of DA deamination in both control and lesioned tissue. These results suggest that glial cells contain a functionally important amount of both type A and B MAO and that these cells play a role in the metabolism of DA.

252.22 COMPARISON OF MODIFIED CARBON PASTE AND CARBON FIBRE ELECTRODES FOR THE ELECTROCHEMICAL MEASUREMENT OF CATECHOLAMINES IN THE PRESENCE OF ASCORBIC ACID. G. A. Newell and E. H. Colhoun*. Department of Pharmacology and Toxicology, The University of Western Ontario, London, Ontario, Canada.

The electrochemical determination of catecholamines in brain has been complicated by interference from naturally-occurring ascorbic acid (AA). To clarify this dilemma, an in vitro comparison of several electrode materials was conducted. The materials used were graphite-nujol epoxy and graphite-nujol paste (Conti, et al. Life Sci. 23:2705, 1978), dodecyl sodium sulphate and stearic acid modified versions of the above and the pyrolytic carbon fibre electrode of Gonon, et al. (Anal. Chem. 53:1386, 1981). The anionic modifiers were incorporated into the graphite mixtures to improve the selectivity for dopamine (DA) over AA. Each electrode type was evaluated by differential pulse voltammetry using both pure solutions of AA, DA and 3,4-dihydroxyphenylacetic acid (DOPAC) and combinations of various concentrations of AA + DA and AA + DOPAC in phosphate-buffered saline. In the non-modified "Conti" style electrodes, AA shifted the half-wave potentials ($E_{\frac{1}{2}}$) to more positive values, depending on the relative concentrations of AA to either DA or DOPAC. The electrodes which had been treated with the anionic modifiers had low sensitivity to both AA and DOPAC, but had high sensitivity to DA. It also appeared that their $E_{\frac{1}{2}}$'s were shifted to more positive values. For AA + DA, only stearic acid paste and the dodecyl sodium sulphate epoxy versions were potentially useful. In all the above treatments, however, the oxidation current for DA or DOPAC varied with AA concentration. The carbon fibre electrode was able to distinguish between AA and DOPAC over a wide range of relative concentrations and showed linear response for a plot of concentration versus current. For DOPAC, the $E_{\frac{1}{2}}$ was always at least 150 mV more positive than for AA, which enables both identification and quantitation of both peaks to be made. These in vitro results indicate that the pyrolytic carbon fibre electrode has the greatest potential for in vivo determination of DA and DOPAC in the presence of AA. (Supported by the Defence Research Board of Canada.)

252.23 METHYLXANTHINES STIMULATE CENTRAL NOREPINEPHRINE METABOLISM IN VIVO. Matthew Galloway* and Robert H. Roth, (Spon: S. Bunney) Pharmacology and Psychiatry, Yale Univ., New Haven, CT 06508

We have previously shown that systemic administration of 3-isobutylmethylxanthine (IBMX) to rats stimulates central noradrenergic function as evidenced by increased levels of the major norepinephrine (NE) metabolite, 3-methoxy-4-hydroxy phenylglycol (MHPG). Others have shown that IBMX (i.v.) can increase cell firing in the locus coeruleus (LC). Furthermore, the effects of IMBX on MHPG levels and cell firing can be reversed by the alpha-2 agonist clonidine, a drug capable of activating presynaptic autoreceptors. To characterize further the interaction between IBMX and NE systems, we have studied the effects of IBMX on NE turnover, DOPA accumulation, and MHPG levels after administration of an adenosine agonist or after dorsal bundle axotomy. In rats treated with IBMX (100 umol/kg, i.p.) and the decarboxylase inhibitor NSD-1015, there was a 50-100% increase in DOPA levels in hippocampus, cortex, cerebellum and olfactory tubercles which was maximal 1.5h after treatment. This effect, which was prevented by clonidine (75 ug/kg), suggests that tyrosine-3-monooxygenase can be activated in vivo by IBMX. When catecholamine synthesis was inhibited by alpha-methyltyrosine (2 hr), IBMX enhanced the depletion of NE in several brain areas suggesting that NE turnover was also increased by IBMX. When NE terminals in the forebrain were dissociated from their cell bodies in the LC by cutting the ascending dorsal bundle, the ability of IBMX to elevate MHPG was still evident when measured 5 or 18 hr after the axotomy. Since adenosine is known to inhibit NE release in several preparations and IBMX is an adenosine antagonist in vitro, the effects of the adenosine agonist 2-Cl-adenosine (2.5 & 5 mg/kg) on MHPG levels were also studied. It was found that 2-Cl-ad alone had little effect on MHPG levels and did not reverse the IBMX-induced increase in MHPG.

These data demonstrate that IBMX is capable of increasing NE synthesis, catabolism, and turnover in vivo. Since the IBMX induced increases in MHPG and tyrosine hydroxylation are still apparent after severing the axon, the effects appear to be a consequence of enhanced release at the nerve terminal and not due primarily to actions elicited at the NE cell body. These data are consistent with the hypothesis that increased functional noradrenergic activity, a characteristic of opiate withdrawal in rats and primates, may give rise to the "quasi-morphine withdrawal syndrome" associated with methylxanthine administration. Supported in part by MH 14092, MH14276, DA 02321 and the State of Connecticut.

252.24 CATECHOL-O-METHYLTRANSFERASE IN HUMAN AND RAT CELLS CHARACTERIZED BY GEL ELECTROPHORESIS AND IMMUNE FIXATION. M.H. Grossman[1], M. Braverman*[1], C.R. Creveling[2], R.M. Weinshilbaum[3], J. Dunnette*[3] and X.O. Breakefield[1]. [1]Dept. Human Genetics, Yale Univ. Sch. Med., New Haven, CT 06510; [2]Lab. Bioorganic Chem., NIH-NIAMDD, Bethesda, MD 20205; [3]Dept. Pharmacology, Mayo Fdn., Rochester, MN 55901.

Catechol-O-methyltransferase (COMT: EC 2.1.1.6) acts to degradatively methylate catechols in all tissues of the body. Several studies have suggested multiple forms of this enzyme, including membrane-bound and soluble forms, and forms differing in apparent MW. Here, rabbit antisera prepared against COMT purified from rat liver and partially purified from human kidney were used to localize the position of immunoreactive proteins in polyacrylamide gels following transfer to nitrocellulose paper. Partially purified COMT from rat liver showed a single immunoreactive band, apparent MW ∿26000, following SDS-PAGE. This same preparation gave two immunoreactive bands after isoelectric focusing under denaturing conditions, pIs 5.2 and 5.3. Similar immunoreactive proteins were observed in crude homogenates prepared from cultured rat hepatoma cells. Analysis of human red blood cell lysates showed a single immunoreactive band on SDS gels, apparent MW ∿26000, and two sets of tightly spaced band on isoelectric focusing gels, clustering at pI 5.3 and 5.6. Similar immunoreactive molecules, presumed to be COMT, were also observed for partially purified COMT from human kidney and homogenates of human neuroblastoma cells (cells from Dr. June Biedler). Studies are underway to characterize immunoreactive proteins in other human and rat cell types, and to map the chromosomal location of human COMT using somatic cell hybrids (in collaboration with Dr. Uta Francke). These studies suggest that in both human and rat cells a single MW species of COMT exists in at least two forms differing in net charge.

252.25 INCREASED STRIATAL DOPAMINE TURNOVER AFTER CHRONIC APOMORPHINE ADMINISTRATION.

Dana M. Vaughn and Richard E. Wilcox
Dept. of Phamacology, College of Pharmacy, University of Texas, Austin, TX 78712.

Chronic apmorphine (APO) administration is followed by a behavioral supersensitivity to the stereotypic effects of challenge doses of APO (Wilcox, Riffee, Chen, Hammett and Smith, Psychopharmacology, 72, 113-115, 1980) but no change in striatal ($3\bar{H}$)-spiroperidol maximum receptor density (Riffee, Wilcox, Vaughn and Smith, Psychopharmacology, in press). These data suggest that presynaptic mechanisms may play a role in the behavioral supersensitivity after chronic APO administration. We evaluated changes in in vivo striatal dopamine turnover after chronic APO or saline administration to test this hypothesis.

Dopamine turnover may be assessed by analysis of the disappearance of dopamine vs. increases in l-dopa after dopa decarboxylase inhibition by NSD1015. APO (30 mg/kg,ip) or saline was administered once daily for 14 days. Three days after the last chronic injection, mice were challenged with saline, spiroperidol (0.002 or 0.01 mg/kg ip) or APO (2 or 5 mg/kg ip). Thirty minutes later NSD1015 (100 mg/kg ip) was administered. The animals were sacrificed 30 minutes after the NSD1015 was given. Striatal dpamine turnover in vivo was assessed by HPLC (reverse phase with electrochemical detection). Major findings were as follows.

1. After chronic APO administration, dopamine turnover in saline/NSD mice was increased by 24% relative to turnover in mice chronically treated with saline. Thus, basal dopamine turnover increased after chronic APO treatment.
2. Spiroperidol challenge (0.002 and 0.01 mg/kg, respectively) produced 7 and 18% increases in dopamine turnover after APO vs. 29 and 53% increases after chronic saline administration. Therefore, after chronic APO treatment, spiperone challenge produced smaller increases in dopamine turnover.
3. APO challenge (2 and 5 mg/kg, respectively) produced 48 and 61% decreases in dopamine turnover after chronic APO injections vs. 43 and 50% decreases after chronic saline treatment. Thus, after chronic APO treatment, APO challenge resulted in greater decreases in dopamine turnover.

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252.26 REGULATION OF SUBUNIT FORMS OF DOPAMINE-β-HYDROXYLASE IN THE PC12 PHEOCHROMOCYTOMA CELL LINE. E.L. Sabban[1,2], M. Goldstein[1] and L. A. Greene[3]*, Departments of Psychiatry[1], Cell Biology[2] and Pharmacology[3], New York Univ. Med. Center, New York, N.Y. 10016.

Dopamine-β-hydroxylase (DβH) is a characteristic enzyme of noradrenergic neurons and adrenal chromaffin cells. DβH is present in both the soluble and membrane bound fractions of adrenal chromaffin granules and in neuronal noradrenergic vesicles.

In this study we examined the regulation of DβH in the PC12 rat pheochromocytoma cell line. PC12 cell monolayer cultures were labelled with ^{35}S-methionine for several hours. DβH was isolated by immunoprecipitation with specific anti-rat-DβH antibodies, analyzed on gradient polyacrylamide slab gels in the presence of SDS, and the labelled protein was detected by flurography. Two subunit forms, with apparent molecular weights of 77K and 73K were observed. They were present in approximately equal amounts. Similar results were obtained by immunoprecipitation from cells labelled with ^{3}H-mannose. The 77K form was localized in a crude membrane fraction, while the 73K form was soluble (presumably within the vesicles). Both fractions possessed DβH enzymatic activity. The soluble form, however, had a specific activity 10-fold lower than the membrane form.

Treatment of the PC12 cells with nerve growth factor (NGF) leads to neurite outgrowth. When NGF-treated cells were labelled with ^{35}S-methionine for several hours, the immunoprecipitate contained almost exclusively the 73K subunit form of DβH. The cells had to be treated for at least 2 days with NGF before the 73K form predominated. The effect was detectable at 10ng/ml NGF and conversion was complete with 30ng/ml of NGF. However, the effect of NGF was not dependent on neurite outgrowth. Thus, it also occurred when the cells were treated with NGF in suspension or when low concentrations of inhibitors of transcription were added to prevent neurite outgrowth. Preliminary pulse chase experiments showed that in the NGF-treated cells the 77K form of DβH may be a precursor for the 73K form.

Insulin (100ng/ml) and epidermal growth factor (1ng/ml) had no effect on the distribution of the DβH subunit forms. However, treatment with dexamethasone (10^{-5}M) or dibutyryl cyclic AMP (1mM) for several days reproduced the effect of NGF and led to synthesis of predominately the 73K form.

The variation in the distribution of the DβH subunit forms elicited by NGF, dexmethasone and dibutyryl cyclic AMP raises the possibility that this phenomenon may play a role in the overall regulation of the enzyme in vivo. Supported by NINDS 06801, NIMH 02717 (M.G.) and NS 16036 (L.A.G.).

252.27 MECHANISMS FOR THE ACTIVATION OF TYROSINE HYDROXYLASE IN THE ADRENAL MEDULLA BY DECAPITATION STRESS. A.W. Tank,* J. Meligeni* and N. Weiner. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, Colorado 80262.

Tyrosine hydroxylase (TH) is activated in the adrenal medulla after decapitation stress (Masserano and Weiner, Mol. Pharmacol. 16: 513-528, 1979). Control activity is measured in adrenal supernatants prepared from rats which have been anesthetized with pentobarbital prior to removal of the adrenals. The mechanism(s) responsible for this in vivo, stress-induced activation of adrenal TH remains obscure. It has been shown that TH can be activated in vitro by cyclic AMP-dependent protein kinase (cA-PK), and that this activation correlates with the direct phosphorylation of the enzyme (Vulliet et al, Proc. Natl. Acad. Sci, U.S.A. 77: 92-96, 1980; Yamauchi and Fujisawa, J. Biol. Chem. 254: 6408-6413, 1979). Thus, it is possible that cyclic AMP-dependent phosphorylation of adrenal TH is responsible for the activation of the enzyme that occurs in vivo. If this hypothesis were true, there should be fewer sites available for phosphorylation in vitro by cA-PK and ATP-γ-^{32}P on the enzyme isolated from stressed rats than on the enzyme isolated from nonstressed rats.

When adrenal supernatants prepared from nonstressed rats are incubated at 30°C with 8-bromo cyclic AMP and an optimal concentration of purified catalytic subunit of cA-PK, TH activity rises rapidly, reaching a peak level after 2 minutes of incubation. When adrenal supernatants prepared from stressed rats are incubated under identical conditions, TH activity increases to a maximal level after 1 minute of incubation. The incorporation of ^{32}P-phosphate into adrenal TH isolated from nonstressed rats follows a similar time course as the activation of the enzyme in these supernatants. However, the time course of ^{32}P-phosphate incorporation into TH isolated from stressed rats does not correlate with the time course of activation of TH in these supernatants; ^{32}P-phosphorylation of TH proceeds at a linear rate for 4 minutes of incubation. The final level of ^{32}P-phosphate incorporation into TH isolated from stressed rats is not significantly different than that incorporated into TH isolated from nonstressed rats. However, the rate of ^{32}P-phosphorylation of TH isolated from stressed animals is less than the rate of ^{32}P-phosphorylation of TH isolated from nonstressed animals. The ^{32}P-phosphorylation of adrenal TH isolated from decapitated rats which proceeds after TH activity has reached its maximum is not due to the loss of unlabeled phosphate from the enzyme followed by the rephosphorylation of the enzyme with labeled phosphate, nor is it due to ^{32}P-phosphorylation of sites on TH other than the site normally phosphorylated by cA-PK. These results suggest that there are the same number of vacant cyclic AMP-dependent phosphorylation sites on adrenal TH isolated from stressed or nonstressed rats. Thus, a mechanism other than cyclic AMP-dependent phosphorylation of TH is involved in the activation of the enzyme that occurs in vivo after decapitation stress. Supported by USPHS grants NS 09199, NS 07927, and AA 03527.

252.28 LOCUS CERULEUS LESION WITH 6-HYDROXYDOPAMINE CAUSES IRREVERSIBLE NORADRENERGIC DENERVATION OF THE CEREBRAL CORTEX. S. I. Harik. Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Lesion of the nucleus locus ceruleus (LC) results in marked depletion of norepinephrine (NE) in the ipsilateral cerebral cortex. Such depletion reaches its maximum at 2 weeks after the lesion and is maintained for 8-10 weeks. Despite continued NE depletion, the increased density of β-adrenoceptors, the increased activity of isoproterenol-stimulated adenylate cyclase, and the abnormalities of cerebral oxidative metabolism that were demonstrated in the denervated ipsilateral cortex 2 weeks after LC lesion all return to normal by 4-8 weeks (Harik et al, J. Neurosci. 1:641, 1981). Possible explanations for this recovery of function include: (i) regeneration of LC neurons, (ii) increased neuronal activity and/or terminal sprouting of the few noradrenergic LC neurons that escape the lesion, and (iii) compensatory changes in other neurotransmitter systems.

To determine whether regeneration and/or increased activity of central catecholamine neurons are viable possibilities, we measured the activities of tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) and levels of NE, dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in samples of the temporoparietal cerebral cortex ipsilateral and contralateral to unilateral LC lesion. LC lesion in adult Wistar rats was performed 2 and 8 weeks earlier by the local stereotaxic microinfusion of 5 μg of 6-hydroxydopamine. Two weeks after LC lesion, ipsilateral cortical NE decreased to 15% ($p < 0.001$), TH decreased to 20% ($p < 0.001$), and DBH decreased to 13% ($p < 0.001$) of the values obtained from the contralateral cerebral cortex. There were no significant differences in DA and DOPAC levels between the 2 sides. Eight weeks after the lesion, ipsilateral NE was still depleted to 18%, TH to 16% and DBH to 21% of values from the contralateral cortex, and DA and DOPAC were barely detectable. These results do not favor regeneration or hyperactivity of remaining LC neurons as viable explanations for recovery. It is likely that functional recovery after chronic LC lesion is mediated by noncatecholaminergic mechanisms.

252.29 SOLUBILIZATION OF A PUTATIVE ANTIDEPRESSANT RECEPTOR (^{3}H-IMIPRAMINE BINDING SITE) FROM HUMAN PLATELETS. A. Davis, J. Morris*, and S.W. Tang. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada M5T 1R8.

^{3}H-Imipramine has been shown to bind to specific, high-affinity sites both on blood platelets and brain membranes, and is believed to label the recognition site for the 5-hydroxytryptamine carrier system. We now report the successful solubilization of this site.

The pharmacological profiles, regional localization, and sodium-ion dependency are very similar for both the uptake and binding systems. To further explore this relationship, we have used digitonin to solubilize the ^{3}H-imipramine binding site from human platelets. No difference in binding characteristics was observed between fresh and outdated platelets; the latter are therefore an excellent tissue source for these studies.

Platelets (72-94 h old) were obtained from the Toronto Red Cross and washed extensively. Solubilization was carried out with 1% (w/v) digitonin final concentration with removal of unsolubilized material by centrifugation at 105,000 g for 60 min. Solubilized binding sites were precipitated by 10% polyethylene glycol and 0.1% bovine γ-globulin carrier protein and assayed by filtration.

The binding site affinity and density remained effectively unchanged upon solubilization. The maximum potency change for any drug was a 4-fold drop in affinity for desipramine. Hill coefficients for all drugs, in both membrane-bound and solubilized preparations were close to unity implying homogeneity of the binding site population. Purification of this binding site is now underway.

	Membrane-bound	Digitonin-solubilized
K_d (nM)	4.95 ± 0.65 (8)	4.9 ± 2.7 (5)
B_{max} (fmol/mg protein)	559 ± 41	537 ± 78
	IC_{50} (nM)	
Imipramine	4	8
Fluoxetine	9	13
Desipramine	102	400
5-Hydroxytryptamine	8,000	4,500
Mianserin	18,000	40,000
Iprindole	23,000	48,000

Dr. A. Davis was supported by the C.K. Clarke Foundation and the Clarke Associates. Dr. S. W. Tang is an Ontario Mental Health Foundation Scholar.

252.30 TETRAHYDROBIOPTERIN ADMINISTRATION TO RHESUS MACAQUES. T.R. Insel,* L. Miller,* M. Sheinin,* J. Aloi,* W. Lovenberg,* D.L. Murphy,* M. Linnoila* (SPON: Christy L. Ludlow). Clinical Neuropharmacology Branch, National Institute of Mental Health, Bethesda, MD 20205.

Tetrahydrobiopterin (BH_4) is an essential cofactor for the first and rate limiting step in the biosynthesis of catecholamines from tyrosine and the biosynthesis of serotonin from tryptophan. We administered BH_4 (dose 15-20 mg/kg) intravenously in a placebo controlled experiment with rhesus macaques (n = 6) to evaluate (1) penetration of peripherally administered BH_4 into primate cerebrospinal fluid (CSF), and (2) effects of BH_4 on catecholamine and indoleamine metabolite levels. Rhesus macaques were studied in restraining chairs with CSF collected by continuous sampling through cervical cannulas.

BH_4 in CSF measured by the acid oxidation method of Fukushima and Nixon (Anal. Biochem. 102, 176-188, 1980) increased in the first 90 minutes and peaked at 3-4 hours following peripheral administration. Peak levels ranged from 3-30 nmoles/ml of CSF, as much as a 700-fold increase over baseline levels. CSF levels of BH_4 returned to baseline at 24 hours.

CSF monoamine metabolites were measured by HPLC with electrochemical detection. Preliminary results suggest minimal mean changes for four animals although considerable inter-individual variation was evident. Urinary excretion of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) also failed to increase significantly after BH_4. Urinary excretion of cortisol showed a trend towards increasing on the active drug day. There was no evidence of behavioral change or change in heart rate following BH_4 administration.

While it appears that BH_4 penetrates into the CSF of primates, these marked cofactor increases appear insufficient to change catecholamine or indoleamine metabolite levels. Nevertheless, the inter-individual variance in the current results suggests that in certain animals or perhaps in states with deficits of cofactor, BH_4 administration might result in neurochemical and behavioral changes.

253.1 OPPOSITE EFFECTS OF CLONIDINE ON THE FLEXOR REFLEX IN ACUTELY-SPINALIZED VS. NON-SPINALIZED RATS. J.H. Kehne*, D.W. Gallager, and M. Davis. (SPON: C.A. Sorenson). Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508.

Clonidine produces an α_1-noradrenergic (NE) mediated excitation of the flexor reflex (FR). In other behavioral paradigms, clonidine produces depressant effects mediated by α_2-NE receptor activation. Importantly, the FR is routinely measured in spinalized preparations, suggesting that clonidine might produce different effects if administered to the non-spinalized animal. The present study sought to compare the effects of clonidine on the FR in acutely-spinalized vs. non-spinalized rats.

The FR was elicited by presenting multiple intensity electrical pulses through electrodes implanted subcutaneously in the base of the hindpaw, and was measured with a force tranducer and polygraph. Rats were either transected at T1-T2 under halothane anesthesia or anesthetized but not transected and two hours later tested for their responses to intraperitoneally-administered clonidine. Clonidine produced opposite effects on the FR in the two preparations. As reported previously, clonidine (0.12-2.0 mg/kg) produced a dose-dependent increase in FR amplitude. In non-spinalized rats, clonidine (.007-2.0 mg/kg) produced a dose-related decrease. The excitatory effect of 0.50 mg/kg clonidine in the spinalized rat was blocked by pretreatment 1/2 hour before testing with 1.0 mg/kg of the α_1-NE antagonist prazosin, and partially attenuated by pretreatment with 10 mg/kg of the α_2-NE antagonist piperoxane. In the non-spinalized rat, the depressant effect of 0.50 mg/kg clonidine was attenuated by piperoxane, but not by prazosin treatment. These data suggest that spinal transection produces a shift in the effects of clonidine on the FR from an α_2-mediated inhibition to an α_1-mediated excitation.

One explanation for the shift in the effects of clonidine on the FR is that transection removes a supraspinal, α_2-mediated inhibitory mechanism that normally masks the excitatory effect of stimulation of α_1-NE receptors. Studies utilizing a rapid reversible blockade of impulse flow by intrathecal infusion of procaine have revealed a shift in the clonidine response from inhibition to excitation. Alternatively, transection might "bring out" α_1-agonist properties of clonidine by inducing a rapid development of supersensitivity. Consistent with this possibility, an increase in the number of 3H-prazosin labelled receptor sites in the lumbar cord, without a change in Kd, was found two hours following transection. Studies are being carried out to distinguish between these possibilities.

253.2 EXCITATORY EFFECTS OF THE VASODILATOR HYDRALAZINE ON ACOUSTIC STARTLE: POSSIBLE NORADRENERGIC MEDIATION. R.L. Commissaris* and M. Davis, Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508.

The acoustic and tactile startle reflexes are affected by many psychoactive drugs. In addition to their effects on neuronal transmission in the central nervous system (CNS), many drugs that alter startle also alter blood pressure. Interestingly, there is a correlation between these two measures, since many drugs that increase startle amplitude also increase blood pressure, either by central actions or by actions in the periphery (at the level of the vasculature). Similarly, many agents which depress startle amplitude also depress blood pressure. Therefore, the purpose of the present study was to further explore the possible relationship between blood pressure and startle amplitude. The peripherally-acting vasodilator hydralazine was chosen because it produces marked hypotension but apparently lacks direct effects on neuronal transmission in the CNS, although it does have marked indirect (compensatory) effects on sympathetic activity.

Male rats were presented with startle-eliciting noise bursts (115 dB; 50 msec duration, 120 sec ISI) for 14 minutes to establish a pre-injection baseline. The animals were then removed from their cages, injected IP with saline or 0.625-10.0 mg/kg hydralazine, and returned to their cages and received startle-eliciting noise bursts for an additional 90 minutes. While saline injections had no effect on startle amplitude, hydralazine surprisingly produced a marked and dose-dependent Increase in startle amplitude (relative to baseline) over the range of doses used.

Since increases in noradrenergic activity are known to increase startle amplitude, it is possible that the increase in startle amplitude observed following hydralazine administration is due to increased central sympathetic (rebound) activity in response to decreased blood pressure. To test this hypothesis, the α_1-antagonist prazosin (0.125-1.0 mg/kg) was administered 10 minutes prior to the administration of 5.0 mg/kg hydralazine in another set of experiments. Prazosin pretreatment significantly attenuated the startle-increasing effects of hydralazine administration.

In conclusion, since the doses of hydralazine used in the present study would produce marked hypotension, these data provide evidence clearly dissociating changes in startle amplitude from changes in blood pressure. Moreover, the data suggest that the acoustic startle reflex, and perhaps other behaviors, may be modified by drug actions occurring outside the CNS, which only indirectly affect neuronal activity in the CNS.

253.3 CORRELATIONS BETWEEN α_1-ADRENERGIC STIMULATION OF ACOUSTIC STARTLE AND α_1-ADRENOCEPTOR OCCUPANCY AND NUMBER. D.I. Astrachan*, M. Davis, and D.W. Gallager (SPON: D.B. Menkes), Dept. Psychiat., Yale Univ. Sch. of Med., New Haven, CT 06508.

The relationship between alterations in α_1-adrenoceptors and behavioral effects of α_1-adrenergic agonists were investigated in a localized region of the rat CNS. Direct infusion of the α_1-adrenergic agonists d-amphetamine or phenylephrine (PE) onto the lumbar cord (intrathecal administration) increased the amplitude of the acoustic startle reflex. The magnitude of this behavioral facilitation correlated highly with the degree of α_1-adrenoceptor occupation measured by 3H-prazosin binding in the lumbar spinal tissue. Maximal potentiation of startle occurred with approximately 30% occupation of the receptors, using either d-amphetamine or PE. Intrathecal administration of 6-OHDA produced a 96% decrease in spinal norepinephrine and markedly enhanced the behavioral response to intrathecal PE as well as the number of α_1-adrenoceptors. The correlation between behavioral and binding changes at various times after the lesion was 0.99. No change in receptor affinity (KD) or receptor occupancy by PE was found after 6-OHDA.

At 6 hours after an initial infusion of PE (which increased startle by about 95%) the same dose of PE was infused again. At this time, PE only increased startle by about 3%. Infusion of the indirectly acting α_1-agonist d-amphetamine also failed to enhance startle in rats given PE 6 hours earlier. However, these same rats still showed marked excitatory effects when infused intrathecally with non-α_1-adrenergic drugs such as the glycine antagonist, strychnine, and the 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine. These results suggest a specific functional desensitization or tachyphylaxis of the α_1-adrenoceptor response following prolonged exposure to PE. At times when this functional desensitization was observed, no significant alterations in 3H-prazosin binding density or affinity were seen. In addition, the correlation between receptor occupation and behavioral responsivity was only 0.11 at 6 hours post PE.

Our data indicate that receptor binding parameters can have predictive value for behavior within a specified time period, especially if localized regions of the CNS, critical to the behavior, are analyzed. However, since these correlations tended to decline with prolonged agonist exposure, the data suggest that: 1) 3H-prazosin binding is not capable of distinguishing active from inactive α_1-adrenoceptive sites; or 2) functional desensitization occurs at a stage beyond the α_1-adrenoceptive recognition site.

253.4 CHANGES IN STRIATAL DOPAMINE RELEASE FOLLOWING VARIOUS STIMULI AS MONITORED BY IN VIVO VOLTAMMETRY. R.W. Keller, Jr., M.J. Zigmond and E.M. Stricker. Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have been using in vivo voltammetry to study the effects of stimuli on striatal dopamine (DA) release in freely moving male Sprague-Dawley rats as measured by surface modified (dodecyl sodium sulfate) carbon paste-epoxy microvoltammetric electrodes (150 µm tip) implanted in the caudate nucleus. Chronoamperometric measurements made at +0.5 V were used to detect extracellular changes in "DA release" which in our measurement included released DA, any co-released ascorbic acid, and any extracellular increase in dihydroxyphenylacetic acid resulting from the release of DA. In addition to observing the reported dose-dependent increase in DA release observed by others with amphetamine and l-dopa, we observed increases in the electrochemical signal following a number of treatments such as electric shock to the tail (74 nAmp increase above baseline with peak at 4 min), placing the animal in an ice water bath (19 nAmp, 7 min), olfactory stimulation by ammonia (21 nAmp, 16 min), i.p. injection of an irritant such as 1 M NaCl (28 nAmp, 20 min), and presentation of food following 24 hr food deprivation (12 nAmp, 22 min). The increase in electrochemical signal appears to reflect DA release since these signals were markedly decreased by pretreating the animal with α-methyl-p-tyrosine (200 mg/kg) or γ-hydroxybutyrolactone (750 mg/kg), drugs which are known to decrease DA release. Other treatments, such as i.p. injection of isotonic saline, placing the animal in a refrigerated chamber, or presenting a cookie to a satiated animal, produced little or no change in the electrochemical signal. Moreover, acute homeostatic challenges such as glucoprivation caused by 2-deoxyglucose (750 mg/kg, ip), hypovolemia caused by polyethylene glycol (5 ml of 30% solution, sc), and hypotension caused by phentolamine (10 mg/kg, sc), all of which provoke marked increases in peripheral sympathetic activity, produced no significant change in the electrochemical baseline. These and other results suggest that DA may be released in the striatum primarily by external stimuli of an intense nature which have a marked onset.

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253.5 NOREPINEPHRINE INFUSED INTO THE MEDIAL PREOPTIC AREA INHIBITS LORDOSIS BEHAVIOR IN FEMALE RATS. J. D. Caldwell* and L. G. Clemens. (SPON: J. Edwards). Dept. of Zoology, Michigan State University, East Lansing, MI. 48824.

The ovarian hormones, estrogen and progesterone, act synergistically in female rats to induce sexual receptivity. Lordosis, a ventral flexion of the back, is a primary component of female receptivity in the rat. The medial preoptic area (MPOA) is thought to play an important role in mediating lordosis behavior. Norepinephrine (NE) is a major chemical constituent of the MPOA (Dahlstrom, A. and K. Fuxe. Acta Physiol. Scand. 64 (Suppl. 247) 39-61 1965). This study evaluated changes in lordosis behavior following NE infusions into the MPOA of ovariectomized, hormone-treated rats.

Infusions of NE into the MPOA of highly receptive females resulted in a significant reduction of lordosis frequency within 5 minutes. Infusions of NE were made through bilateral cannulae chronically implanted in the MPOA. All infusates were given in 1 µl artificial cerebrospinal fluid (aCSF) infused over 1 minute. Doses of 2, 5 and 10 µg/µl NE resulted in significant reductions in lordosis behavior within 5 minutes. No general debilitation was seen and lordosis frequency returned to control levels 20 minutes after infusion. There was no reduction of lordosis as a result of NE infusions into the medio-basal hypothalamus. In an attempt to block the short-latency inhibitory effect of NE in the MPOA three noradrenergic antagonists were tested with 2 µg of NE. The beta-noradrenergic antagonist propranolol or one of the alpha-antagonists yohimbine or phentolamine were infused simultaneously with the NE. The antagonist concentration was 5 µg/µl. Only the alpha-antagonist yohimbine blocked the significant drop in lordosis seen with NE infusions. This suggested that norepinephrine might act on alpha-noradrenergic receptors to inhibit lordosis. To test this animals were infused with two doses of the alpha-agonist clonidine (0.5 & 1 µg/µl) in the MPOA. Both doses significantly reduced lordosis frequency as compared to aCSF infused controls at 5-20 minutes after lordosis.

In order to test if NE had any facilitative effects on lordosis, six doses of NE (0.1-10 µg/µl) were infused into unreceptive estrogen-treated rats. These rats failed to show a significant increase in lordosis over aCSF infused controls.

These data are consistent with the concept that norepinephrine can act at medial preoptic alpha-receptors to inhibit lordosis behavior.

253.6 ALTERATIONS IN AMPHETAMINE-INDUCED STEREOTYPY THROUGH ACUTE LESIONS OF SUBSTANTIA NIGRA. L.P. Gonzalez. Dept. of Physiology & Biophysics. Univ. of Ill. Med. Ctr., Chicago, Ill. 60680.

Stereotypy induced by high systemic doses of amphetamine (Amph) has been related by many investigators to the ability of this drug to increase the release of dopamine in the caudate nucleus. Since Amph-stimulated dopamine release in the caudate is blocked by acute lesions of the nigrostriatal pathway (Von Voigtlander and Moore, J. Pharmacol. Exp. Ther., 1973, 184, 542-552), the mechanisms by which Amph acts to produce stereotypy may be dependent upon intact nigrostriatal impulse flow. In contradiction to this suggestion, my laboratory has previously reported that inhibition of nigrostriatal impulse flow through local application of drugs into substantia nigra pars compacta (SN) does not alter Amph-induced stereotypy (Gonzalez and Ellinwood, Brain Res., 1981, 208, 223-226). The studies reported here examined the effects on Amph stereotypy of acute electrolytic lesions of SN and the nigrostriatal projections.

Groups of male Sparague-Dawley rats received chronic bilateral implants of twisted, bipolar electrodes in SN. After recovery from surgery doses of 0, 3.0, and 6.0 mg/kg of d-Amph were administered intraperitoneally to different groups of subjects, and the acute response determined. Stereotypy was scored at five minute intervals through use of a rating scale, and was also quantified objectively by means of an electronic motility transducer (Ellinwood et al., Pharmacol. Biochem. Behav., 1981, 15, 627-631). Thirty minutes after systemic Amph, the electrodes of half of the subjects were connected to an electrical lesion maker and a bilateral lesion of SN performed. Groups of control subjects were also attached to the lesion maker, but no lesion was performed. Animals were then returned to the observation chambers and stereotypy measured for an additional 30 minutes. The acute response of these same animals to Amph was observed a second time five days after this initial session. Animals were then sacrificed and the placement and extent of lesions determined histologically.

The systemic administration of Amph was followed by dose- and time-dependent alterations in behavior, with the higher dose (6.0 mg/kg) producing a more intense stereotypy than the lower dose (3.0 mg/kg). Acute lesions of the nigrostriatal pathway did not alter Amph-induced stereotypy. The lesioned animals, however, were significantly less sensitive to Amph when administered five days after the lesion. These results indicate that an acute interruption of nigrostriatal impulse flow is not sufficient to alter Amph stereotypy, but that chronic interruption of this pathway, presumably with subsequent degeneration of terminals in the striatum, does alter the behavioral response to Amph.

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253.7 A COMPARISON OF THE EFFECTS OF LIMBIC VERSUS STRIATAL DOPAMINE DEPLETIONS ON SPONTANEOUS AND DRUG-INDUCED BEHAVIOR. R. J. Carey. VA Medical Center, Syracuse, N. Y. 13210.

Rats were subjected to two types of unilateral or bilateral 6-hydroxydopamine lesions of brainstem dopamine containing neurons. One set of lesions produced severe losses of striatal dopamine (< 5% control level) and the other lesion reduced limbic dopamine (< 10% control level). Bilateral loss of limbic dopamine reduced locomotor activity, increased catalepsy scores, but did not affect rigidity. This lesion also modified the influence of dopaminergic agonists on locomotor activity. d-Amphetamine (0.5 and 1.0 mg/kg) had no effect on activity, but apomorphine (0.125 and 0.25 mg/kg) markedly increased locomotor activity. In contrast, the bilateral loss of striatal dopamine increased indices of both catalepsy and rigidity, but did not affect locomotor activity, nor the effects of amphetamine (increase) or apomorphine (decrease) on locomotor activity. Unilateral 6-hydroxydopamine lesions of striatal and limbic dopamine neurons produced spontaneous turning toward the dopamine depleted hemisphere for several days postoperative. After one month postoperative, the two unilateral lesions differentially affected the rats' response to drug-induced circling. The unilateral loss of striatal dopamine produced the typical turning responses to amphetamine (1.0, 2.0, 4.0 mg/kg) and apomorphine (0.25, 0.5, 0.75, mg/kg), that is, turning toward the lesion with amphetamine and away from the lesion with apomorphine. This was not the case for the unilateral depletion of limbic dopamine which produced turning toward the lesion with both amphetamine and apomorphine treatments. This study indicates that limbic dopamine has an important role in motoric function which is distinct from the role of striatal dopamine.

253.8 THE EFFECTS OF QUIPAZINE AND METERGOLINE ON LOCOMOTOR ACTIVITY IN A FAMILIAR AND NOVEL ENVIRONMENT, R.J. Beninger and B.L. Hahn*. Dept. Psychology, Queen's University, Kingston, K7L 3N6, Canada.

A large number of studies investigating the effects on locomotor activity of various manipulations of the brain's serotonin systems has led to many inconsistent findings; thus, both increased and decreased activity as well as no change has been reported following depletion of serotonin with parachlorophenylalanine, intraraphe injections of 5,7-dihydroxytryptamine or electrolytic lesions of the raphe nuclei. Besides employing different techniques for altering brain serotonin, these studies differed in the amount of apparatus preexposure given to the animals, length of test session, size of apparatus and level of illumination. To test the possibility that the effects of altered serotonin neurotransmission on locomotor activity might be affected by interexperiment differences in the level of familiarity that animals have with the apparatus, this variable was systematically manipulated in groups of rats treated with the serotonin agonist, quipazine or the serotonin receptor blocker, metergoline.

The activity monitoring apparatus consisted of 6 Plexiglas chambers (41x50x37 cm) housed in ventilated, insulated, dimly lit outer boxes. Each chamber had a grid of 7 infrared emitters and detectors arranged at a height of 5 cm and monitored by a single board microcomputer, thus allowing for continuous recording of locomotor activity. Ninety-six male rats were subdivided into 2 groups, one (preexposed condition) of which received 5 30-min preexposure sessions in the apparatus, one session per day; the other (novel condition) group received equivalent handling. Then each condition was subdivided into 8 groups and given 3 30-min test sessions on the next 3 days; 30 min prior to each session groups received i.p. injections of quipazine (0, 2.5, 5.0, 10.0 mg/kg) or metergoline (0, 2.5, 5.0, 10.0 mg/kg).

Separate analyses of variance for each drug within each condition were carried out with intrassession time, test sessions and dose as the variables analysed. For the quipazine groups, results revealed no significant (sig.) drug effect or interactions in the novel condition but a sig. drug by time interaction in the preexposed condition; post hoc tests showed a dose-dependent decrease in activity early in the session. For the metergoline groups, the drug had no sig. effect in the preexposure condition but sig. reduced activity in a dose- and time-dependent manner in the novel condition. These results show that the effects on locomotor activity of compounds affecting serotonergic neurotransmission interact with the level of novelty of the test apparatus and with the duration of testing. Interexperiment differences in these variables may account for inconsistencies previously reported in the literature concerning the role of serotonin in locomotor behavior.

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253.9 REINSTATMENT OF ORIENTING BEHAVIOR IN RATS WITH SUPERIOR COLLICULUS LESIONS BY D-AMPHETAMINE. G.C. Midgley* and R.C. Tees. Dept. of Psychology, Univ. of Western Ontario, London, Ont., N6A 5C2 and Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The involvement of the nigrotectal pathway in stereotyped behaviors and locomotor activity has been assessed by combining superior colliculus (SC) lesions and increased dopamine transmission produced by administration of d-amphetamine (Pope et al., Psychopharmacology, 70, 1980). In this study we attempted to investigate the role of the nigrotectal pathway in the expression of orienting behavior by this combination of lesion and dopamine transmission manipulations.

Male rats from the Long-Evans strain received either sham operations (SH), large bilateral SC lesions (SC), small lesions of the superficial layers of the SC (SP), small lesions of the deep layers of the SC (DP), or lesions of the striate cortex (ST). The animals received either 1 mg.kg d-Amphetamine or vehicle injections 20 min prior to testing. Orienting behavior elicited by apparently moving or stationary light displays, habituation of orienting behavior, and recovery of orienting behavior with changes in the visual stimuli were assessed by examining the rats' tendency to interrupt an ongoing behavior and perform appropriate head and postural adjustments.

Amphetamine pretreatment had a small but reliable effect on the habituation of orienting behavior for the SH animals. These animals interrupted their ongoing behavior and oriented to the lights on more trials than the vehicle-SH animals. Vehicle injected SC and DP animals did not respond to the light displays employed in this study. However, Amphetamine injected SC or DP animals did respond to the lights. The topography of their orienting behavior, rate of habituation, and recovery of orienting with changes in the light display was comparable to the SH animals.

These findings are interpreted in light of the observation that impairment of orienting behavior also follows damage to the neostriatal dopamine system and is reinstated with injections of the dopamine receptor stimulant apomorphine (Marshall et al., J.C.P.P., 94, 1980). They are suggested as support for a view of SC lesion impaired orienting behavior as a disturbance of sensory attention and emphasizes the interaction of the SC and other neural systems in processes mediating the direction of attention towards impinging stimuli (Midgley & tees, Exp. Brain Res, 41, 1981).

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253.10 UNILATERAL NEUROTOXIC LESIONS OF CORTICAL DOPAMINERGIC, BUT NOT SEROTONINERGIC PATHWAYS INDUCE AN ASYMMETRICAL BEHAVIORAL RESPONSE IN THE RAT. R. S. Black*and R. G. Robinson. (Spon: J.B. Wirth), Dept. of Psychiatry & Behavioral Sciences, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Right middle cerebral artery ligation, as well as right cortical injections of the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) in the rat leads to a period of spontaneous hyperactivity, accompanied by 60-70% reductions in norepinephrine and dopamine in both ipsilateral and contralateral brain samples. In contrast, left lesions produce no such changes. This study uses desmethylimipramine (DMI) combined with 6-OHDA and 5,7 dihydroxytryptamine (5,7-DHT) to produce lesions specific to dopamine and serotonin pathways, examining the role of these neurotransmitters on post-lesion hyperactivity. Animals were housed in running wheel cages for 3 weeks prior to operation and baseline activities were recorded. One hour before neurotoxin injection animals were given DMI 25 mg/kg. Animals were anesthetized and given a frontoparietal craniotomy. A cannula was positioned about 9 mm anterior and 2 mm dorsal to ear bar zero without damaging vessels. Two µg of 6-OHDA and either 2 or 4 µg of 5. 7-DHT was injected in saline/ascorbate vehicle into either the right or left cortex 1 mm below the surface. Five animals received vehicle injections into the right hemisphere. Postoperative activity was collected for 28 days and then the animals were sacrificed and their brains dissected into ipsilateral and contralateral samples of lesioned cortex, caudate nucleus, uninjured posterior cortex, locus coeruleus, substantia nigra, and brainstem, which included the raphe nuclei. Samples were analyzed for norepinephrine, dopamine, and serotonin using high pressured liquid chromatography with electrochemical detection. After 5,7-DHT injections with DMI pretreatment, right and left lesioned animals at both doses slowly returned to baseline activity levels without becoming hyperactive despite 60-70% reductions in serotonin concentrations in the frontal cortex, posterior cortex, and median raphe, without reductions in dopamine and norepinephrine. Animals who received 6-OHDA into the right hemisphere became hyperactive on about days 7-9 to 150% of baseline. The left hemisphere injected animals became slightly hyperactive (about 120% of baseline) on about day 21, a highly significant ($p<0.01$) difference. Biochemical analyses have not yet been completed on this group. This study demonstrates that post-lesion hyperactivity is probably not related to the destruction of serotoninergic neurons, and suggests that dopamine may be involved in addition to norepinephrine. It also suggests that this asymmetrical hyperactivity is neurotransmitter specific and not related to nonspecific aspects of the brain lesions.

253.11 MESOSTRIATAL DOPAMINE SYSTEMS AND EATING AND DRINKING EVOKED BY HYPOTHALAMIC STIMULATION: DIFFERENCES BETWEEN THE DOMINANT AND NON-DOMINANT HEMISPHERES. Guy Mittleman* and Elliot S. Valenstein. Psychology Department and Neuroscience Lab, University of Michigan, Ann Arbor, MI 48109.

Data from several experiments suggest that eating and drinking evoked by electrical stimulation of the lateral hypothalamus (ESLH) are dependent on brain catecholamines and specifically dopamine (DA). Evoked eating can be blocked by intraventricular infusions of 6-Hydroxydopamine (6-OHDA) and also systemic injections of the DA receptor blocker, haloperidol (Phillips & Fibiger, Behav. Biol., 9:749-754, 1973; Phillips & Nikaido, Nature, 258:750-751, 1975; Rowland, Marques & Fisher, Physiol. Behav., 24:273-281, 1980). Because these techniques are relatively unspecific, the neural pathways most critical for ESLH-evoked eating and drinking have not been identified. The purpose of the present experiments was to determine the specific role of the mesostriatal DA system in evoked eating and drinking. Exp. I: Long-Evans male rats were implanted with bilateral hypothalamic electrodes and 2 pairs of bilateral cannulae oriented in an anteromedial-posterolateral plane in the striatum. All animals received 3 tests for evoked eating and drinking on both electrodes using an ascending series of stimulus intensities. The 12 animals that exhibited evoked eating during each test session received a unilateral infusion of 6-OHDA through 1 pair of cannulae. The results proved to be very variable. Three animals died, two or more weeks after the infusion; the behavior of 3 was unimpaired; while the evoked behavior of 6 animals was disrupted for at least 30 days. In the second experiment we investigated whether some of the variability in our results could be attributed to the reported asymmetry in the mesostriatal DA system (Glick et al., Biochem. Pharm., 23:3223-3225, 1974). Exp. II: In this experiment Long-Evans rats were first tested for amphetamine-induced rotational behavior to estimate the degree of lateralization of the mesostriatal DA system. They were then implanted with bilateral electrodes and cannulae and tested as in Exp. I. Animals exhibiting evoked consummatory responses received unilateral infusions of 6-OHDA into the striatum of either their dominant or non-dominant hemisphere. These animals were tested for evoked behavior at least once each week for up to 30 days. Of the animals lesioned on the dominant hemisphere, 80% have shown disruption of evoked behavior, while only 25% of the rats receiving a lesion in the non-dominant hemisphere showed any deficit.

These results suggest that the mesostriatal DA system in the dominant hemisphere may play a major role in the eating and drinking evoked by ESLH from either side of the brain.

253.12 NOVEL CORTICAL LOCALIZATION OF A LATERALIZED BEHAVIOR IN THE RAT: RELATIONSHIP TO NORADRENERGIC INNERVATION. G. D. Pearlson, K. L. Kubos* and R. G. Robinson. Department of Psychiatry and Behavioral Sciences, Johns Hopkins University, Baltimore, MD 21205

Previous research has documented that small cortical aspiration lesions in the right hemisphere of the rat lead to spontaneous locomotor activity and to widespread bilateral NE depletion: identical left cortical lesions produce neither phenomenon (Pearlson, G. D. and Robinson, R. G., Brain Research, 218:233, 1981). NE innervation of lateral rat cortex proceeds caudally from the frontal pole (Morrison, J. H., Molliver, M. E. and Grzanna, R., Science, 205:313, 1979).

300-gram male Sprague-Dawley rats received a 2 mm diameter single focal ablation or sham lesion in the left or right cerebral cortex using a micro-pipette at König and Klippel stereotaxic AP coordinate 9650, 8620, 3990 or 1400 µ. Spontaneous daily activity compared to preoperative baseline was assessed for 30 days postoperatively; animals were then sacrificed for histology, or NE determination by HPLC in several bilateral cortical and subcortical regions. Right cortical lesions produced a graded effect on bilateral NE depletion: the more anterior the lesion the greater the fall in NE ($R = .97$, $p < .01$). A similar graded effect was seen with the hyperactivity following right-sided lesions; the more posterior the lesion the less the degree of hyperactivity (factorial ANOVA for lesion location on right $F = 5.22$; DF 3, 142; $p < .01$). Left operated animals showed no graded effects on activity or NE.

These results suggest a novel pattern of cortical localization for a lateralized behavior, that is, a graded phenomenon across the entire hemisphere. This pattern might be expected from the underlying anatomy of neurotransmitter (NE) pathways.

253.13 SEX DIFFERENCES IN THE EFFECTS OF EARLY HANDLING ON BRAIN AND BEHAVIORAL ASYMMETRIES. Dianne M. Camp*, Jill B. Becker and Terry E. Robinson (SPON: C.M. Butter). Psychology Dept. and Neuroscience Lab., University of Michigan, Ann Arbor, MI 48109.

Behavioral asymmetries have been related to asymmetries in brain organization. In rats the development of some of these asymmetries is influenced by early environmental manipulations. For example, Sherman et al. (Br. Res., 192, 1980) reported that the development of spatial preferences in male rats is influenced by early handling experience. In this experiment we explored the possibility of sex differences in the effects of early handling on the development of brain and behavioral asymmetries. Eight litters of Holtzman rats were reduced at birth to 10 pups, with equal or nearly equal numbers of males and females. Each litter was assigned to either a handled (H) treatment group or a non-handled (NH) control group. From day 1 through day 20 post-natally each pup in the H group was placed for 3 min into an individual compartment of a box containing wood shavings. NH pups were not disturbed during this time period. On day 21 all litters were weaned and group housed on a reverse day/night cycle until day 51, after which they were housed in individual cages. At approximately 2½ months of age each animal was randomly placed in one of the corner squares of an open field (16 squares each measuring 30 cm^2). The corner in which the rat was placed was initially surrounded by an L-shaped barrier. After 10 sec the barrier was removed and the initial direction of movement recorded. Subsequent behavioral activity was recorded for a total of 3 min. All animals received 4 tests/day for 4 consecutive days. All testing was performed under red light conditions during the night part of the rats' day/night cycle. At the end of the experiment, animals were killed by decapitation and the right and left nucleus accumbens and right and left striatum dissected from the brain. Dopamine (DA) levels were measured using an HPLC with electrochemical detection. In the open field test we found that females were more active than males ($F=18.5$, $p<.04$). However, there was also a significant sex X handling interaction ($F=23.2$, $p<.01$). Whereas H males were more active than NH males there was no difference in activity between the H and NH female groups. Early handling also influenced the left-right distribution of DA in the nucleus accumbens of male rats. In only H males the DA levels in the right nucleus accumbens were significantly higher than in the left ($t=2.17$, $p<.04$). No similar asymmetry was found in striatal DA levels. Lastly, only H males and NH females showed a significant behavioral asymmetry in the open field, both being left-biased. We conclude that: 1) experiences early in life may influence the development of brain and behavioral asymmetries in rats, and 2) early handling has different effects on brain and behavioral asymmetries in male and female rats.

253.14 FUNCTIONAL ASYMMETRY IN THE NIGROSTRIATAL DOPAMINE SYSTEM. Terry E. Robinson and Jill B. Becker. Psychology Dept. and Neuroscience Lab., University of Michigan, Ann Arbor, MI 48109.

The rotational behavior observed in rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal dopamine (DA) system has been widely used as a functional index of striatal DA activity. It is usually thought that such rats circle ipsilateral to the lesion (ipsiversive) when injected with amphetamine (AMPH), regardless of which side of the brain is depleted of DA. However, Glick and his colleagues have suggested there is an endogenous asymmetry in the nigrostriatal DA system, and therefore side may be an important consideration. To investigate this hypothesis we first tested intact rats for AMPH (1.2 mg/kg)-induced rotational behavior. On the basis of this screening one hemisphere was defined as being "dominant" (D) for rotational behavior (the side contralateral to the dominant direction of rotation), and the other side as "nondominant" (ND). The animals were then pretreated with DMI and injected with 6-OHDA (8µg in 4µl) into either the D or ND substantia nigra. After at least 2 weeks recovery all rats were again tested for AMPH-induced rotational behavior. The pattern of rotational behavior observed after the 6-OHDA lesion depended on 2 factors: 1) the extent of the striatal DA depletion and, 2) the side of the lesion. For animals with a >90% striatal DA depletion ($\bar{X}$=98.5%) the side of the lesion had very little effect. Of those rats with a ND-sided lesion, 9/9 rotated ipsilateral to the lesion. Of their total rotations, 100% were ipsiversive. Of rats with a D-sided lesion 9/10 rotated preferentially to the ipsilateral side. Of their total rotations 93.7% were ipsiversive. In contrast, the side of the lesion had a dramatic effect in rats with a <90% striatal DA depletion ($\bar{X}$=67.0%). The majority of rats (9/11) with D-sided lesions and a <90% DA depletion rotated in the contraversive direction (i.e., in the opposite direction of all other groups), despite the fact that some of these animals had 80-90% DA depletions. These animals made only 24% of their total rotations ipsilateral to the lesion. However, animals with a <90% DA depletion and ND-sided lesions were like animals with a >90% DA lesion. All of them (n=8) showed ipsiversive rotation, making 96.5% of their total rotations in that direction. The unique effect of D-sided lesions was most evident when rats were tested with low (1.2 mg/kg), rather than high (3-5 mg/kg) doses of AMPH. These data suggest that functionally the endogenous asymmetry in the nigrostriatal system must be very robust. When only 10-20% of the DA input to the D hemisphere was left intact most animals still turned away from that side. Obviously, the D hemisphere cannot compensate when it is nearly totally destroyed, as seen in rats with >90% depletions.

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253.15 BEHAVIORAL EFFECTS OF SOME NOVEL APORPHINES IN RATS WITH 6-HYDROXYDOPAMINE-INDUCED LESIONS OF THE NUCLEUS ACCUMBENS OR CAUDATE NUCLEUS. E.A. Jackson*, J.L. Neumeyer and P.H. Kelly. Dept. of Physiology and Biophysics, University of Southern California School of Medicine, Los Angeles, CA 90033, and (J.L.N.) Dept. of Medicinal Chemistry and Pharmacology, Northeastern University, Boston, MA 02115.

The behavioral actions of some novel aporphines have been examined in rats with selective unilateral 6-hydroxydopamine (6OHDA)-induced destruction of nigrostriatal dopamine neurons, and in rats with bilateral 6OHDA-induced destruction of mesolimbic dopamine neurons. Dopaminomimetics such as apomorphine in these animal models elicit circling behavior and locomotor activity respectively. In animals with unilateral nigrostriatal lesions (-)-2,10,11-trihydroxy-N-n-propylnoraporphine (TNPA) and (-)-10,11-methylene-dioxy-N-n-propylnoraporphine (MDO-NPA) elicited weak, but prolonged, contraversive circling, whereas (-)-2,10,11-trihydroxyaporphine (2-OH.APO) was inactive. In animals with bilateral destruction of mesolimbic dopamine neurons TNPA and MDO-NPA elicited a strong stimulation of locomotor activity, while 2-OH.APO was inactive. The results suggest that TNPA and MDO-NPA, but not 2-OH.APO, exert central dopaminomimetic effects in vivo. The results are also consistent with previous data indicating that N-propyl substitution of aporphines causes a relative enhancement of activity in animal models which emphasise effects at mesolimbic rather than striatal dopamine receptors.

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254.1 THE EFFECTS OF METHYLPHENIDATE ON THE FIRING RATES OF NIGRAL DOPAMINE CELLS: INTERACTION WITH BODY TEMPERATURE, Tong H. Lee* and Everett H. Ellinwood, Jr., M.D. (SPON: H. K. H. Brodie). P.O. Box 3870, Duke University Medical Center, Durham, North Carolina 27710.

Using an extracellular recording technique, we have investigated: (1) effect of methylphenidate on the firing rates of dopamine (DA) cells in the substantia nigra zona compacta (SNC), and (2) interaction of its effect with changes in body temperature. Following catheterization of the jugular vein under chloral hydrate anesthesia (400 mg/kg/, i.p.), rats were mounted on a Kopf stereotaxic apparatus, and a 3 mm burr hole drilled in the skull in an area overlying the SNC. Recordings were made using glass-coated tungsten electrodes with impedence of 9-12 M ohm at 1000 Hz. The DA cells in the SNC were identified by their location, characteristic firing pattern, and wave form. After baseline data were collected for 5-10 minutes, increasing doses of methylphenidate were administered every 3 minutes. The effect of the drug was reversed by haloperidol. In order to examine the effect of body temperature changes on methylphenidate dose-response, rectal temperatures of some of the animals were allowed to be raised from 36-37°C to 38.5°C and subsequent baseline activity recorded for additional 5-10 minutes before administering 4.0 mg/kg methylphenidate. The drug responses were recorded for 10-15 minutes before injection of haloperidol.

Dopamine cells in the SNC could be separated into two groups based on their sensitivity to methylphenidate. At the cumulative dose of 4.0 mg/kg, i.v., the more sensitive cells were inhibited below 20% of their baseline activity, wheras the other group of cells had firing rates ranging 40-85%. Histological examination of the recording sites revealed that the sensitive cells were more anteriorly located.

We have preliminarily determined that, under hyperthermia, there was little change in baseline activity of nigra DA cells. However, some of DA cells surprisingly increased their firing rates after methylphenidate while the majority of cells were, as expected, significantly inhibited. In all cases, the effect of methylphenidate was reversed by subsequent haloperidol. These cells are probably dopaminergic based on their locations in the SNC and their firing characteristics. More extensive data on this temperature sensitivity including interactions among various putative neurotransmitters (e.g., serotonin) will be discussed.

254.2 ACTIVITY OF DOPAMINE-CONTAINING VENTRAL TEGMENTAL AREA NEURONS IN FREELY MOVING CATS. D. W. Preussler and M. E. Trulson. Lab. for Neurobiology, Univ. of Texas at Dallas, Richardson, TX 75080.

Dopamine-containing neurons in the ventral tegmental area (VTA) project primarily to the limbic system, including the olfactory tuberacle, nucleus accumbens and lateral septal regions. Some of these neurons also project to the neocortex, particularly the frontal cortex and cingulate gyrus. The meso-limbic and meso-cortical dopamine systems are believed to be involved in various psychological functions, and disorders of these systems have been implicated in certain psychiatric disorders, such as schizophrenia. Although numerous studies have been directed at elucidating the function of the meso-limbic and meso-cortical systems, relatively little is known about the role these neurons serve in behavior regulation. One obstacle to furthering our understanding of VTA neurons is that all electrophysiological studies have been performed in animals that were anesthetized and/or immobilized. Therefore, we recorded the activity of dopamine-containing VTA neurons in freely moving cats, while concurrently monitoring behavior. Unit activity was recorded by means of movable 32 or 64 u dia insulated Nichrome wires (see Brain Res. 163, 1979, 135-150 for complete methodology). Dopamine-containing VTA neurons were initially identified on-line by their unusually wide action potential (2-4 msec) and characteristic slow-bursting firing pattern with progressive decreases in spike amplitude or a slow and regular discharge pattern. This identity was later confirmed by the nearly total inhibition of unit activity by low doses of apomorphine (1 mg/kg, i.p.), and a pronounced excitation (+ 47.1%) by low doses of haloperidol (0.5 mg/kg, i.p.), as well as by histological analysis, i.e., all units were located in the densely dopaminergic VTA region. During quiet waking (i.e., no overt movement) VTA neurons displayed a slow, somewhat irregular activity ($\bar{X}$= 3.8 ± 0.7 spikes/sec). Unit activity showed no significant change from quiet waking during slow-wave sleep ($\bar{X}$= 3.5 ± 0.6 spikes/sec) or REM sleep ($\bar{X}$= 3.6 ± 0.5 spikes/sec). In this regard, VTA neurons are very much like dopamine-containing pars compacta neurons of the substantia nigra, which also show no change in activity across the sleep-waking cycle. However, unlike nigral units, VTA neurons showed no significant change in activity during active waking (i.e., when the cat is showing overt behavior). Recording the activity of VTA units in freely moving animals has several advantages over the anesthetized preparation. For example, correlations between drug-induced behavioral and unit changes can be investigated, as well as the effects of various sensory stimuli. Furthermore, the effects of manipulations such as prolonged stress can be evaluated. Finally, potential circadian changes in unit activity can be studied, since it is possible to record these neurons continuously for more than 24 hours using the present technique.

254.3 UNIT ACTIVITY OF SEROTONIN-CONTAINING NEURONS IN THE NUCLEUS CENTRALIS SUPERIOR IN FREELY MOVING CATS. Kurt Rasmussen, James Heym and Barry L. Jacobs. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

The activity of serotonin-containing neurons in the mesencephalic dorsal raphe nucleus (DRN) and the medullary nucleus raphe pallidus (NRP) has been examined under a variety of conditions in freely moving cats (Brain Res. 163:135-150, 1979; Heym et al., Brain Res. in press). These data indicate that the serotonin-containing neurons in these two nuclei have similar general characteristics, but that important differences also exist between them. In an effort to extend our analysis of the brain serotonergic system, the present study examined the characteristics of serotonergic and non-serotonergic cells in the region of the nucleus centralis superior (NCS) of adult freely moving cats. Single unit activity was recorded utilizing a movable microwire technique previously described in detail (Brain Res. 163:135-150, 1979). Two bundles, each consisting of three 32 and three 64 µm diameter insulated nichrome wires, were implanted 1.5 mm above and behind the NCS (P 2.7, L 0.0, H -1.3) at an angle 45 posterior to vertical. Electrodes were also implanted for recording the EEG, EOG, and dorsal neck EMG. Serotonergic neurons were initially identified on-line by their slow and regular spontaneous activity which was similar to that previously reported for serotonergic cells in the DRN and NRP. In general, the activity of NCS cells was more similar to DRN than NRP cells. Discharge rates of NCS serotonergic cells were highest during waking (2.2 ± 0.3 spikes/sec, mean ± s.e.m., n = 15), decreased during slow wave sleep (1.2 ± 0.3 spikes/ sec), and were lowest during REM sleep (0.5 ± 0.2 spikes/ sec). These cells responded with a mean decrease in unit activity of approximately 50% to administration of a low dose of the serotonin specific agonist 5-methoxy-N,N-dimethyltryptamine (5-MeoDMT 50 µm/kg, i.m.). All cells responded with a short latency (∿ 40 msec), short duration (∿ 30 msec) excitation to visual and auditory stimulation, except for a subset of these cells (n = 4) that responded with a short latency (∿ 10 msec), long duration (100-200 msec) inhibition. Non-serotonergic cells in the area had either much more irregular or much faster firing rates, did not display their lowest firing rates in REM sleep, and did not respond with decreases in activity after administration of 5-MeoDMT. These data indicate that the serotonin-containing cells of the DRN, NRP, and NCS are relatively homogeneous, although the NCS is more similar to its anatomical neighbor, the DRN. The similarity of serotonergic unit activity in the DRN and NCS implies that their reported differential roles in behavior are mediated by their differential projections, rather than by differences in their unit activity per se. (Supported by NIMH Grant MH 23433).

254.4 EFFECTS OF DIAZEPAM ON DOPAMINE-CONTAINING SUBSTANTIA NIGRA UNITS IN FREELY MOVING CATS. V. M. Trulson and M. E. Trulson. Lab. for Neurobiology, Univ. of Texas at Dallas, Richardson, TX 75080.

Diazepam has potent anti-anxiety effects at low doses, but produces motor impairment, including ataxia and atonia, at high dose levels. One central neural system which has been demonstrated to be involved in motor control is the dopaminergic nigral-striatal system. Accordingly, we investigated the effects of various doses of diazepam on the activity of single dopamine-containing cells in the pars compacta of the substantia nigra in freely moving cats, to determine whether this drug alters the activity of dopaminergic neurons. Nigral unit activity was recorded by means of movable 32 and 64 u dia insulated Nichrome wires (for complete methodology, see Neurosci.Lett. 26, 1981, 183-188). Dopamine-containing substantia nigra units were initially identified on-line by their unusually wide action potential (2-4 msec) and characteristic slow-bursting firing pattern with progressive decreases in spike amplitude. This identity was later confirmed by histological analysis, i.e., all units were located in the densely dopaminergic pars compacta of the substantia nigra. During quiet waking (i.e., no overt movement), nigral units displayed a mean dischage rate of 3.9 ± 0.5 spikes/sec. There was no significant change in unit activity during slow-wave sleep (3.5 ± 0.4 spikes/ sec) or REM sleep (3.8 ± 0.5 spikes/sec). However, unit activity was significantly increased by 20.5% during active waking (i.e., when the animal was displaying overt movement). Administration of low doses of diazepam (i.e., 0.5-1.0 mg/kg, i.p.) produced no significant change in the discharge pattern or rate of nigral units. However, high doses of the drug (10 mg/kg), which produced ataxia and atonia, significantly decreased nigral unit activity by 28.1%. Furthermore, the firing pattern was also altered, showing a very rhythmic discharge, without the occurrence of bursting observed in the non-treated animal. In addition, neuronal activity remained unchanged when the animal displayed brief episodes of phasic movement. A centrally acting muscle relaxant (mephenesin, 200 mg/kg, i.p.) produced very similar effects on nigral unit activity, while a peripherally acting muscle relaxant (dantrolene, 100 mg/kg, i.p.) produced no significant change in nigral unit discharge pattern or rate. There are prominent reciprocal connections between the substantia nigra and striatum, which is an integral part of the central motor system. The presence of GABAergic neurons in both the striatum and substantia nigra are of particular interest, in view of the apparent close association between benzodiazepine receptors and GABAergic neurons. These data suggest that diazepam may exert its effects on motoric behavior, in part, by altering the dopaminergic input to the striatum.

254.5 CHLORAL HYDRATE ANESTHESIA ALTERS THE RESPONSIVENESS OF DORSAL RAPHE NEURONS TO PSYCHOACTIVE DRUGS. M. E. Trulson and V. M. Trulson. Lab. for Neurobiology, Univ. of Texas at Dallas, Richardson, TX 75080.

Single unit recordings from serotonin-containing raphe neurons have greatly increased our knowledge of the physiology and pharmacology of the central serotonergic system in mammals. These studies were initially conducted exclusively in anesthetized and/or immobilized rats. Recently, however, we have sucessfully recorded single raphe neurons in freely moving cats (e.g., Brain Res. 163 1979, 135). Interestingly, there have been several notable examples of differential effects of psychoactive drugs on the activity of raphe neurons in chloral hydrate anesthetized rats vs freely moving cats. To determine whether these various effects are due to species differences, route of drug administration, or to the anesthesia, we examined the effects of several psychoactive drugs on raphe unit activity in freely moving cats and in cats that were anesthetized with chloral hydrate. Administration of LSD (75 µg/kg, i.p.) to anesthetized rats has been shown to produce a total inhibition of raphe unit activity (Gallagher and Aghajanian, J. Pharm. Exp. Ther. 193, 1975, 785), while the same dose of LSD administered to freely moving cats suppressed raphe unit activity by only 67.3%. However, when the cats were first anesthetized with chloral hydrate (350 mg/kg, i.p.), 75 µg/kg of LSD produced a total inhibition of raphe unit activity. Alpha adrenoceptor antagonists have also been shown to dramatically suppress the activity of raphe cells in anesthetized rats, e.g., phenoxybenzamine (3 mg/kg, i.v.) produced a nearly total inhibition of raphe unit activity (Baraban and Aghajanian, Neuropharm. 19, 1980, 355). Administration of phenoxybenzamine (10 mg/kg, i.p.) to freely moving cats, on the other hand, produced no significant change in raphe unit discharge rate. When the cats were anesthetized, however, phenoxybenzamine (10 mg/kg) produced a total inhibition of unit activity. In another previous study using chloral hydrate anesthetized rats (Gallagher Eur. J. Pharm. 49, 1978, 133) it was reported that diazepam (8-12 mg/kg, i.v.) produced no significant change in raphe unit activity. We found that diazepam produced a dose-dependent decrease in the discharge rate of raphe neurons in freely moving cats, from no significant change at 0.5-1.0 mg/kg, i.p., to a nearly total suppression of unit activity at a dose of 10 mg/kg. However, when the cats were first anesthetized with chloral hydrate, raphe units showed only a small reduction (-17.6%) in activity following 10 mg/kg of diazepam. These data demonstrate the importance of conducting such drug studies in awake, freely moving animals. Very different results were obtained in anesthetized vs freely moving animals, and, presumably, the results obtained in freely moving animals are far more relevant to the issue of human drug use.

254.6 SINGLE-UNIT RECORDING OF PRESUMED DOPAMINE-CONTAINING NEURONS IN THE VENTRAL TEGMENTAL AREA OF FREELY-MOVING CATS. G. R. Christoph*, R. J. Leonzio* and K.S. Wilcox* (Spon: L. G. Davis). Neurobiology Group, E. I. du Pont de Nemours & Co., Glenolden, PA 19036.

Five adult female cats were used to study the activity of single neurons in the ventral tegmental area of unanesthetized freely-moving subjects. The neurons of particular interest in this region are dopamine-containing cell bodies of the A10 cell group. Under pentobarbital anesthesia each cat received a chronic implant according to previously described methods (Steinfels, G., et al., Life Sci., 29, 1435-42, 1981). The implant included a miniature microdrive system with 14-16 strands of 62 and 32 micron insulated wires (impedance=0.4-1.0 megohm) arranged in two bundles. The electrode tips of one bundle were stereotaxically positioned dorsal to the ventral tegmental area (coordinates: AP=3.5, ML=1.1, DV=-2.5). Recording sessions began 10 days after surgery. A cable and counterweight system connected the individual microwires to conventional single unit recording and counting equipment. The microwires were advanced ventrally 40-320 microns per week in 40-80 micron steps. Over 300 single neurons in the ventral tegmental area were studied over a period of 5 mo. The vast majority of single neurons were characterized by positive-negative action potentials with spike durations < 1msec and fast unit discharge rates (10-40 spikes/sec). In general, these neurons greatly increased their activity during specific movements such as head turning. A subgroup of 21 cells had slower firing rates (0.5-6 spikes/sec) and relatively long duration action potentials (2msec). Neurons in this subgroup are candidates for A10 dopamine-containing neurons. The activity of these neurons was not clearly related to specific movements, although firing rates were 20-40% greater during sitting/standing epochs than during lying-down/sleep periods. In preliminary pharmacological tests with the slow-firing, wide waveform subgroup of cells, apomorphine (0.25-1.0 mg/kg, i.p.) slowed unit discharge rates in 2 of 3 cells tested. The reduction in firing rate occurred at the onset of stereotyped behavior (limb flicks, head bobbing, abortive grooming). The behavioral and electrophysiological effects of apomorphine were reversed by haloperidol administration (0.5-1.0 mg/kg, i.p.). Haloperidol alone caused behavioral sedation and increased firing rates by 20-30% in 3 of 6 cells tested. Additional behavioral and pharmacological correlates of neuronal activity in the ventral tegmental area will be described.

254.7 INTRACRANIAL SELF-STIMULATION DOES NOT ALTER DOPAMINE METABOLISM. T.J. McCown, T.C. Napier, and G.R. Breese. Biol. Sci. Res. Ctr., U.N.C. Sch. Med., Chapel Hill, N.C., 27514

Many investigations have presented converging, indirect pieces of evidence that dopamine (DA) neurons serve a primary, if not critical, role in the maintenance of intracranial self-stimulation (ICSS) behavior (Wise, Life Sci. 22: 535-542, 1978). This conclusion arises mainly from the observation that pharmacological or surgical decrements in DA transmission also causes decrements in ICSS behavior. However, motor impairment also results from such treatments, making clear interpretations difficult (Fibiger, Ann Rev Pharmacol Tox., 37-56, 1978). The following study measures the concentrations of DA and dihydroxyphenylacetic acid (DOPAC) in the olfactory tubercles (OT), nucleus accumbens (NA), and caudate (CD) after ICSS to evaluate its effects on DA metabolism.

Rats were implanted with bipolar electrodes just rostral to the substantia nigra (3190A., 1.3L.,-2.8V.) This placement was previously shown to support quantifiable electrical stimulation of nigro-striatal DA neurons (McCown, et al. Pharmacologist, 1982). Five groups of rats were established: implanted, but not tested; trained and not tested; rats showing maximal response rates; those showing 50% maximal response; and those demonstrating minimum response rates. Once the animals were trained and the rate of responding stabilized, they were tested in the appropriate grouping for 50 min., and immediately sacrificed by decapitation. The animals in the groups which did not involve ICSS were sacrificed at appropriate times following implantation. The OT, NA and CD were dissected out and frozen. DA and DOPAC concentrations were then measured.

A comparison of implanted versus unimplanted sides within animals was non-significant in the OT, NA or CD, as well as in the unstimulated control groups. Furthermore, no differences were observed between any of the groups in the OT, NA, or CD. Both DA and DOPAC concentrations were the same in animals that were only implanted as compared to any of the animals receiving ICSS. These findings suggest that DA activity in these three brain areas is not correlated with ICSS behavior.

254.8 REWARD SUMMATION FUNCTION ANALYSIS OF THE EFFECTS OF NUCLEUS ACCUMBENS INJECTION OF A DOPAMINE RECEPTOR BLOCKER ON HYPOTHALAMIC SELF-STIMULATION IN RATS. J.R. STELLAR, A. KELLEY,* D. CORBETT.* Dept. of Psych. and Soc. Rel., Harvard University, Cambridge, MA. 02138.

Previous work from many laboratories has indicated that the ascending midbrain dopamine systems play a major role in the elaboration of the rewarding effects of lateral hypothalamic stimulation. In this regard, the nucleus accumbens, a target for the A10 dopamine neurons, has been implicated as one site of action (Wise, R.A., Pharma. Biochem. & Behav., 13:213-223, 1980). However, the behavioral measurement techniques employed were often unable to yeild much quantitative information as to the extent of the reward degradation and, in some cases, were unable to adequately separate effects on motor performance from those on reward.

The reward summation function (RSF) technique, in particular, and psychophysical measurement in general (Gallistel, Shizgal and Yeomans, Psych. Review, 88:228-273, 1981), offer an improvement in both these areas. Briefly, an RSF was generated by measuring the running speed of rats in a runway for a range of conditions of reward, produced by varying the number of square-wave pulses in the reward burst. Duration of the reward burst was always kept at 1.0 seconds. The resulting RSF was analyzed into two properties: the locus of rise (i.e. number of pulses required to reach half maximal performance) and the asymptotic level (i.e. maximum performance). The results under control conditions were compared to those following a 90 second infusion of 0.5 ug/0.5 ul α-flupenthixol bilaterally into the nucleus accumbens. Decreases in the asymptote of the RSF indicate impairments of motor performance ability. Increases in the locus of rise of the RSF represent independent quantitative decreases in the pulse effectiveness of the lateral hypothalamic stimulation in generating reward. For example, if locus of rise doubled, pulse effectiveness was cut in half by the drug.

In all cases, accumbens injections resulted in a significant increase in the locus of rise to a higher number of pulses. This increase represents a mean loss of reward pulse effectiveness of 62% (std error=10%; range=48% to 82%) for the five animals tested. Also, a significant decrease in asymptote occured, indicating simulatneous decreases in motor performance. It would appear that the blockade of a single dopamine projection (accumbens) produces both reward and performance effects when measured with a sensitive behavioral technique.

254.9 SELF-STIMULATION DERIVED FROM NON-DOPAMINERGIC FIBER TRACTS. Roberto A. Prado-Alcalá and Roy A. Wise. Physiol. Dept., Sch. Med., Natl. Univ. México, México, D.F., 04510 and Psychol. Dept., Concordia Univ., Montreal, P.Q., Canada H3G 1M8.

Intracranial self-stimulation (ICSS) is readily observed when electrodes are implanted in dopaminergic nuclei and their associated efferent fiber tracts and terminal fields. Lesions of these structures and pharmacological interference with dopaminergic synaptic activity produce significant reductions in ICSS. These data support the hypothesis that ICSS is critically dependent upon activation of dopaminergic neurons. There are, however, only a few systematic experiments where attempts to elicit self-stimulation from cerebral regions lacking dopamine have been made.

In the present study the capacity for supporting ICSS of three non-dopaminergic fiber tracts was determined: the anterior commissure (AC), the olfactory tract (OTr) and the corpus callosum (CC). Using moveable electrodes, implanted in male hooded rats, it was possible to study the reinforcing properties of these fiber tracts as well as of the neighboring tissue. Rate of responding was assessed at currents ranging from 60 µA down in 2 µA steps to the lowest current that would sustain responding (threshold).

ICSS was observed in twenty-two of the forty-four CC stimulation sites, in five of the eight OTr placements and in the four cases where the AC was stimulated. After testing the CC the electrodes were lowered and the tissue immediately below it (within 250 µm) was also tested. There were no significant differences in response rates between the CC and the dorsal striatum nor between the CC and the dorsal septum. ICSS thresholds differed significantly between the latter two brain areas, but not between the CC and the dorsal striatum. Increments, decrements or no changes in thresholds and pressing rates were found when the OTr and the AC were stimulated after stimulating the olfactory tubercle and the nucleus accumbens, respectively.

These results suggest that the reinforcing properties of ICSS are not necessarily dependent upon the direct activation of dopaminergic systems, and further support the idea that the activation of the ascending dopaminergic systems does not represent the first link in the process of brain stimulation reward.

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254.10 INTRACRANIAL SELF-STIMULATION FROM THE AMYGDALA OF THE RAT: THE EFFECTS OF 6-HYDROXYDOPAMINE-INDUCED DEPLETIONS OF CATECHOLAMINES AT THE SITE OF STIMULATION. R. M. Clavier and D. Wen*. Dept. of Biopsychology, Clarke Institute of Psychiatry, Toronto, Ont., Canada, M5T 1R8.

An electrode-cannula system was used to elicit intracranial self-stimulation (ICSS) from the amygdaloid region in rats to test the behavioral effects of local infusions of the neurotoxin 6-hydroxydopamine (6-OHDA) into the brain area surrounding the electrode tip. ICSS responding was allowed to stabilize over a period of at least 10 days. 24 hrs after the last ICSS trial, the animals were given injections of pargyline (Sigma; 50 mg/kg, i.p.), and 30 minutes later they were anesthetized with pentobarbital and placed into a stereotaxic apparatus. A 31-guage injection cannula was loaded with 6-OHDA solution, and attached via polyehtylene tubing to a Sage infusion pump. The injection cannula was then fitted into the guide cannula in the electrode. An amount of 5 µg/2.5 µl 6-OHDA (expressed as the base) dissolved in 0.15 M NaCl, and containing 0.2 mg/ml ascorbic acid, was injected over a 15 minute period. The injection cannula was left in place for 5 minutes after the infusion. 24 hrs after the infusions, the animals were tested for ICSS at the same stimulation parameters as prior to the infusions. Daily testing for ICSS continued for a period of 2-3 weeks. 24 hrs after the last post-infusion trial, the brains of these rats were processed for aluminum-formaldehyde-induced fluorescence of catecholamines. Specifically, the animals were injected with 100 mg/kg pargyline (i.p.), and 3 hrs later, anesthetized with pentobarbital. Intracardiac perfusion of 250 mls of ice-cold 4% paraformaldehyde dissolved in a phosphate buffer was followed immediately by removal, blocking, and sectioning the brains (30 µm) using a Vibratome and a bath of Tyrodes buffer. The sections were then incubated in a 1.7% solution of aluminum sulfate (pH 4), mounted onto slides, and air-dried. These slides were stored in an evacuated desiccator overnight, and exposed to formaldehyde vapours (75%) at 80°C for 60 minutes. This permitted the assessment of depletion of catecholamines from the area of the electrode tip, as compared with the corresponding contralateral amygdaloid area. In some rats, there was an apparent complete depletion of fluorescence in the area of the electrode tip. Every rat in this study showed complete behavioral recovery in the sense that at the termination of testing, ICSS rates were at, or above, pre-infusion levels. This finding supports previous results that question the necessity of catecholamine presence and/or release in the immediate vicinity of ICSS electrode tips.

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254.11 ALTERATION OF INTRACRANIAL SELF-STIMULATION IN MICE FOLLOWING INESCAPABLE STRESS. Robert M. Zacharko, Wayne J. Bowers*, Larry Kokkinidis*, and Hymie Anisman. Dept. Psych., Carleton University, Ottawa, Ontario K1S 5B6, CANADA.

It is well established that controllable and uncontrollable shock will differentially influence performance in tasks involving aversive motivation. Likewise only uncontrollable shock will result in depletion of norepinephrine (NE) in various brain regions. Moreover, uncontrollable shock has been shown to alter dopamine (DA) activity in the arcuate nucleus, nucleus accumbens, and mesolimbic frontal cortex but not in other DA rich areas such as the substantia nigra. In addition to the motoric and cognitive consequences of uncontrollable stressors, it has been suggested that reductions in motivation or in the reinforcement value derived from particular responses may be responsible for the behavioural deficits induced by uncontrollable shock. Unfortunately evaluation of motivational processes in aversive paradigms is compromised by the motoric effects of stress treatments. In the present experiment we determined the effects of controllable and uncontrollable shock on responding for intracranial self-stimulation (ICSS) as a possible indicant of motivational changes. Given that catecholamine (CA) activity is altered by stress in the hypothalamus and nucleus accumbens but not in the substantia nigra, it would be expected that reduction of reward and hence ICSS would be restricted to the former two regions. Ten days following implantation of bipolar electrodes, baseline rates of responding for ICSS were established over a 1-week period in a nose-poke task (Kokkinidis & Zacharko, 1979). Animals were matched in triplets on the basis of response rate and exposed to either 60 escapable shocks (150uA), yoked inescapable, or no shock. Immediately thereafter and at intervals of 24-hr and 168-hr, all animals were retested for ICSS. Whereas escapable shock produced a slight increase of responding, exposure to an identical amount of inescapable shock provoked marked reductions of responding for brain stimulation at all intervals. Paralleling the CA alterations induced by stress, these reductions occurred exclusively from the lateral hypothalamus and nucleus accumbens, whereas responding from the substantia nigra was unaffected by the stress treatment. The fact that the effect of uncontrollable stress on ICSS was region specific suggests that factors such as general arousal or motoric changes were not responsible for the performance changes. Moreover the fact that escapable stress was without effect on performance indicates that controllability over the stressor was fundamental to the performance change. Taken together these data indicate that uncontrollable stress may influence motivational/reinforcement processes and implicate the lateral hypothalamus and the nucleus accumbens in this respect.

254.12 EFFECTS OF CAFFEINE AND AMPHETAMINE SO_4 ON SINGLE UNIT ACTIVITY IN CAUDATE NUCLEUS. Kenneth Hirsh, Jesse Forde* and Dorothy T. Chou. General Foods Corp. Tech. Ctr., Cranbury, NJ 08512

Caffeine is known to increase locomotor activity and mood. The brain nigrostriatal dopaminergic system has been suggested to play a role in regulation of these functions. Therefore, we studied the effects of caffeine, as well as amphetamine, on single neurons in the caudate nucleus of urethane (1.5g/kg) anesthetized cats in an effort to explore the involvement of this dopaminergic pathway in the CNS actions of these two agents. Single unit activity was recorded extracellularly (platinum-iridium microelectrodes) from the dorsomedial portion of the caudate nucleus. Caffeine (1.0-2.5mg/kg), amphetamine (0.1-0.2mg/kg), and haloperidol (1-4mg/kg) were given intravenously. In some experiments using multiple-barrel glass microelectrodes to study the mechanism of caffeine's action, dopamine and amphetamine were administered microiontophoretically, and caffeine was applied via micropressure ejection. The results show that caffeine, 1.0-2.5mg/kg i.v. and amphetamine, 0.1-0.2mg/kg i.v. markedly decreased the firing rates of caudate neurons. Though the depressant effects of caffeine and amphetamine were observed to be blocked in some cases by haloperidol, the effects were not found consistently mainly due to the marked action of haloperidol itself on caudate neuron activity. It was also observed that microiontophoretic application of both dopamine and amphetamine, as well as micropressure ejection of caffeine, depressed the firing rate of caudate neurons. Our data suggest that caffeine and amphetamine may activate the nigrostriatal pathway, thus decreasing caudate neuron activity via increasing dopamine release from nerve endings. Previous neurochemical data indicating that caffeine and amphetamine significantly increased high K^+-induced dopamine release from rat corpus striatum synaptosome support this conclusion (1). These findings all suggest that caffeine-induced elevations of locomotor activity and mood may, at least in part, be mediated through activation of the nigrostriatal dopaminergic system.

1. Chou, D.T. et al Fed. Proc. 41:1062, 1982

255.1 RAPID INFUSION OF INTRADUODENAL GLUCOSE INCREASES SUBSEQUENT FOOD INTAKE. Paula J. Geiselman and Donald Novin. Department of Psychology and Brain Research Institute, University of California Los Angeles, CA 90024.

We have demonstrated in rabbits that oral ingestion of a small volume of glucose (10 ml of a .3M solution) increases later chow intake and that bilateral subdiaphragmatic vagotomy potentiates this hyperphagic response. We further report that glucose infused intraduodenally at a slow rate may have a suppressive effect on food intake; but, when infused at a rapid rate, glucose produces a dramatic increase in food intake.

Female New Zealand rabbits were implanted with duodenal cannulae and infused (10 ml/3 kg BW) with a .3M glucose solution and a .15M NaCl solution presented in randomized order. Seven rabbits received infusions at a slow rate (1 ml/min), and ten received the infusions at a fast rate (3 ml/min). After infusion food intake was monitored continuously for four hours.

When infused at the slow rate (1 ml/min), glucose produced a significantly smaller first meal than did the slow infusion of saline. During the first hour postinfusion, the mean meal size following glucose infusion was less than that after saline.

In contrast, when infused at the fast rate (3 ml/min), glucose produced an enhancement of food intake. During the first half hour following glucose infusion, mean feeding rate (g/min), mean meal size, and total food intake were significantly greater than when measured following saline infusion. Compared with the saline condition, the rabbits nearly doubled their food intake during the first half hour after fast infusion of glucose. Throughout the four-hour measurement period, there was no compensation for the increased food intake found in the first half hour postinfusion.

We then investigated the effect of volume of infusant delivered at 3 ml/min. Six rabbits were infused intraduodenally with 10, 20, and 30 ml/3 kg BW of .3M glucose and .15M NaCl solutions. Following infusion of each of the three volumes of glucose, the results were similar to those reported above for the fast infusion condition. Thus, once the rate of infusion was increased sufficiently to produce the food enhancement effect, further increases in volume had no discernible effect.

Based on these results, we hypothesize that glucose produces hunger when it arrives in the duodenum quickly and is absorbed at a rapid rate. Hence, one might eliminate the hunger-stimulating effect of glucose by making dietary manipulations that would slow the rate at which the food arrives in the duodenum and is absorbed.

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255.2 FAT METABOLISM AND HUNGER, R.Carpenter* and S.P.Grossman, Dept. of Behavioral Sci., Univ. of Chicago, Chicago, Il. 60637.

During the first 10 to 20 days of streptozotocin diabetes and during recovery from insulin-induced and diet-induced obesity negative correlations were observed between food intake and plasma levels of the fat metabolites: free fatty acids (FFAs), glycerol, and ketone bodies. Fat utilization appears to suppress hunger mainly through increased plasma FFA levels. When plasma FFA levels were elevated by chronic intravenous infusion of a triglyceride emulsion, the food intake of intact control rats decreased substantially. Intravenous infusion of 4.5 grams of glycerol (10% in water) per day caused extreme elevations of plasma glycerol to levels (6.0 to 9.0 mM/l) much higher than observed during early diabetes or reversible obesity but caused only small decreases in food intake. Elevation of plasma ketones by infusion to levels seen in moderate diabetic ketosis (2.0 to 5.0 mM/l) also caused only small decreases in food intake.

During early STZ diabetes the negative correlation between plasma FFA levels and caloric intake is even better if urine glucose loss is considered. Hyperphagic diabetic rats eat about 90 calories per day more than controls but lose about 20 grams of glucose in their urine. Thus, caloric intake corrected for glucose excretion is normal in hyperphagic diabetic rats. When the corrected caloric intake of hyperphagic diabetic rats is normal, their plasma FFA levels are also normal. During early diabetes plasma FFA levels are greatly elevated but decline as body fat is lost and diabetic hyperphagia appears. Friedman (JCPP 92:109-117,1978) suggested that the fat metabolites suppress hunger during diabetes; Wirtshafter and Davis (Science 198:1271-1273,1977) suggested that plasma glycerol acts as a lipostatic signal. Our results suggest that plasma FFA levels may be the most important signal of fat utilization.

255.3 GLUCOSE AND FATTY ACID METABOLISM IN BRAIN LOCI OF FED AND FASTED RATS. T. R. Kasser*, A. Y. Deutch and R. J. Martin* (SPON: R. K. Thomas). Depts. of Foods and Nutrition, and of Psychology, Univ. of Georgia, Athens, GA 30602

The objective of this study was to determine if there were regional differences in brain uptake and oxidation of glucose and fatty acid in fed and fasted rats.

In vivo substrate uptake: The method of analysis was the brain uptake index which expresses in vivo brain uptake of a ^{14}C-labeled compound in relation to $^{3}H_2O$ uptake. After a 24 hour fast or normal ad libitum intake, rats were anesthesized and fitted with a carotid cannula. Rats received 0.2 ml of test infusate. The infusate was a Krebs-Ringer bicarbonate buffer (1/2 Ca^{+2}) containing 10.0 mM glucose, 1.0 mM palmitate, 2% bovine serum albumin, $^{3}H_2O$ and either (^{14}C-1) palmitate or (^{14}C-U) glucose. Fifteen seconds after infusion, the rat was decapitated, brain removed and coronal slices obtained. Brain areas ipsilateral to the infused carotid were dissected and placed in scintillation vials containing tissue digester. Radioactivity was determined using dual lable counting procedures. Glucose uptake was similar among cortical, hypothalamic and midbrain areas of fed rats. With fasting, glucose uptake in cortical areas remained similar to fed rats. However, glucose uptake was 50% and 75% lower in hypothalamus and midbrain areas of fasted rats compared with fed rats. Fatty acid uptake in the hypothalamus and midbrain was greater than cortical uptake in fed rats. Hypothalamic and midbrain fatty acid uptake was 2-3 times higher in fasted compared with fed rats.

In vitro substrate oxidation: Rates of glucose and palmitate oxidation in various brain loci were examined by using radioactive substrates and monitoring the production of $^{14}CO_2$ during the incubations. Rats were gradually acclimated to the consumption of their normal daily intake within a 2 hour period. The day of the experiment, rats were sequentially given their food and each sacrificed 2 hours after receiving their meal. Caudatoputamen (CP), septum (SP), ventral lateral (VLH) and medial hypothalamus (VMH), and substantia nigra (SN) were dissected with the aid of magnification. Glucose oxidation rates were similar among the brain loci and were not different in specific loci of fasted and fed rats. Palmitate oxidation rates in VLH, VMH and SEP were greater than values obtained in CP and SN of fed rats. With fasting, rates of palmitate oxidation were increased by 45% in VLH. Fasting did not significantly alter fatty acid oxidation in the other neural sites examined.

These data supported the concept that central neurological areas may monitor fatty acid metabolism as a component of food intake and energy balance.

255.4 FOOD INTAKE (FI) EFFECTS OF INTRAPERITONEAL INJECTIONS OF 2-DEOXY-GLUCOSE (2DG), INSULIN (I) AND GLUCAGON (G) IN LIVER (L) DENERVATED (D) AND SHAM (S) OPERATED RATS. L. L. Bellinger and F. E. Williams*, Dept. of Physiology, Baylor College of Dentistry, Dallas, TX 75246.

The L has been hypothesized to contain neuronal glucoreceptors that send information to the CNS influencing FI. Previously we (Physiol. Behav. 26:663, 1981) reported that LD had no effect on the FI of the rats. It was subsequently suggested by others that LD rats might eat normally but still show deficits to certain metabolic challenges. We addressed this issue recently (Fed. Proc. 41:1003, 1982) and further explore it here. Male Sprague Dawley rats were kept on a 12:12 hr. light:dark schedule, lights out 1230 hr. Rats were LD or S operated (body weights at surgery: LD, 201.5±3.5g; S, 198.3±3.8g). Thereafter daily FI or BW of the groups did not vary significantly. The rats were then injected (all tests started at 0800 hr.) with saline (SAL) or 2DG 150 mg/kg and cumulative FI (corrected for spillage) recorded 1/2, 1, 2, and 3 hrs later. FI was increased by 2DG over SAL at 1 hr. (LD 0.4±0.2 vs 1.4±0.3g, <0.01; S 0.5±0.2 vs 1.4±0.3g, P<0.05); 2 hr. (LD, 0.5±0.2 vs 1.6±0.2g, <0.05; S, 0.7±0.2 vs 1.8± 0.3g, <0.05) and 3 hr. (LD, 0.9±0.3 vs 1.7±0.2g, NS; S, 1.2±0.4 vs 2.1±0.2g <0.05). The FI consumed after 2DG did not differ significantly between LD and S. The rats were then tested as above but with I (0.75 IU/kg and 1.5 IU/kg). FI was increased by I over SAL. I 0.75 IU/kg dose: 1 hr. (LD, 0.5±0.2 vs 1.5±0.3g, <0.05; S, 0.7±0.2 vs 1.6±0.3g, <0.05); 2 hr. (LD, 0.7±0.2 vs 1.6± 0.3g <0.01; S, 1.3±0.3 vs 1.7±0.3g, <0.01); 3 hr. [$F(3,46)=2.4$, $P>0.05$]. I 1.5 IU/kg dose: 1/2 hr. (LD, 0.3±0.1 vs 1.1±0.3g, <0.01; S, 0.2±0.1 vs 0.9±0.2, <0.05); 1 hr. (LD, 0.5±0.1 vs 2.4±0.4, <0.01; S, 0.6±0.2 vs 1.9±0.2, <0.01); 2 hr. (LD, 0.5±0.2 vs 2.8± 0.3, <0.01; S, 0.6±0.2 vs 2.2±0.3, <0.01); 3 hr. (LD, 0.5±0.2 vs 3.0±0.2g, <0.01; S, 1.1±0.2 vs 2.4±0.3g, <0.01). The FI consumed after I did not differ significantly between LD and S. Next the rats were given a liquid diet, sweetened condensed milk (Borden) diluted 50% with water, for one hour starting at 0800 hr. After familiarization to the diet (7 days), the rats were injected with SAL or G (540 ug/kg or 800 ug/kg) and initial meal size recorded. Compared to SAL, G decreased meal size in both groups (G 540 ug/kg dose: LD, 9.0±1.1 vs 7.1±1.1 ml, <0.03; S, 11.4±1.2 vs 8.7± 0.8 ml, <0.01; % of SAL, LD, 77.3±9.6; S, 77.1±3.8. G 800 ug/kg dose: LD, 5.8±0.7 ml, <0.01; S, 5.7±0.7 ml, <0.01; % of SAL, LD, 68.7±8.2, S, 50.4±6.7 N.S.). The data do not indicate abnormal FI responses of LD rats to metabolic challenges. Supported in part by BCD Research Funds.

255.5 INSULIN LEVELS, SUBDIAPHRAGMATIC VAGOTOMY AND FEEDING IN THE RAT. D.A. VanderWeele, J.A. Granja,* J.A. Fiene,* J. Favero* and D.A. Deems*. Dept. of Psychology, Occidental College, Los Angeles, CA 90041.

Typically, insulin has been used to stimulate food ingestion and body weight gain in rats. If more moderate and prolonged elevations are produced, however, decreased food ingestion and body weight gain can be observed. We induced nine rats to become mildly diabetic by the injection of streptozotocin (STZ) into the tail vein (20 - 22 mg of STZ per animal). All animals developed mild hyperglycemia and glycosuria; the glycemic levels ranged from 148 to 304 mg/dl and glycosuria was between ½ and 2% - Ames Clinitest Diagnostic). All animals were then assessed for food and water consumption and body weight changes over the next five weeks. Animals showed a significant increase in consumption measures ($p < .01$) and a nonsignificant decrease in body weight ($p > .10$); mean increase in food consumption was 3.4 g, in water intake was 23.4 ml and mean decrease in body weight was 8.3 g. The mild hyperphagia produced was attributable to an increase in mean meal size.

All subjects were then treated with osmotic minipumps (Alza) delivering 2.5 to 4.0 U regular insulin per 24 hr. The minipumps were implanted in the peritoneal cavity under Brevital anesthesia. When the animal recovered from anesthetic, consumption and body weight measures were resumed for a ten-day period. Six animals treated in this manner began to decrease food and water intakes while reversing the body weight loss. After ten days, food intake had returned to pre-STZ levels while water intake remained significantly elevated ($p < .05$). Body weight increased with minipump treatment and was significantly elevated above pre-STZ levels by Day Eight of treatment ($p < .05$).

In a final study, eighteen rats were implanted with minipumps delivering 2.5 U regular insulin per 24 hr after recovering from subdiaphragmatic vagotomy. Food ingestion had been measured before vagotomy, during recovery and again after the animals had fully recovered and were eating at pre-vagotomy levels. Raised insulin had no effects upon food ingestion in this condition. We conclude on the basis of these three studies that impaired insulin release reduces satiability (Study One), restoring insulin levels normalizes satiety without significantly altering water intake (Study Two) and vagotomy reduces hyperinsulinemia's effects on eating.

255.6 Gastric-distention induced catalepsy in recovering lateral-hypothalamic damaged rats. S. Shoham, B.F. Knight, and P. Teitelbaum. University of Illinois at Urbana-Champaign, Champaign, Ill. 61820.

In stage I of recovery from bilateral lateral hypothalamic (LH) damage, rats do not eat (aphagia) or drink (adipsia) and exhibit catalepsy (a behavioral state characterized by the maintenance of static stable equilibrium even while in awkward postures and resistance to passive displacement by bracing). These rats must be kept alive by intragastric tube-feeding of liquid diet. With recovery, aphagia, adipsia, and catalepsy gradually disappear. The rats accept wet palatable food but they will not eat enough to maintain body weight (stage II). These rats are eventually able to regulate their body weight and survive by eating wet food (stage III). In stage IV they maintain themselves on dry food and water. When a large load (15cc) of liquid diet was given to recovering LH damaged rats (n=15) who showed little or no catalepsy, all movement stopped and catalepsy was immediately induced. Tests for catalepsy were: clinging to two horizontal bars and bracing in response to horizontal displacement. In a cataleptic stage I rat tube-feeding did not enhance catalepsy. In stages II and III longer clinging times and increased bracing scores were recorded following tube-feeding. In stage IV only one rat showed the tube-feeding induced catalepsy phenomenon. In some rats a certain dissociation in catalepsy appeared since clinging and bracing were not always simultaneously enhanced or induced by tube-feeding. Starvation appears to enhance the tube-feeding effect. Fluctuations in body weight were not correlated with the effects of tube-feeding. Induced catalepsy occured following an intragastric water load (15cc) suggesting that stomach distention is a sufficient cause of the induced catalepsy although the role of nutritious ingredients in the liquid diet cannot be ruled out. LH damage depletes dopamine. DA depletion results in catalepsy. Since we could not induce catalepsy in normal rats by gastric distention, there may be an association between DA depletion and gastric distention induced catalepsy. Gastric distention may induce a general organized motor inhibition as a component of a short term satiety mechanism. This inhibition in LH damaged rats may be via suppression of the residual DA system, resulting in catalepsy.

255.7 HYPOPHYSECTOMY OR SPINAL CORD TRANSECTION DOES NOT BLOCK INHIBITION OF FOOD INTAKE BY BOMBESIN. J. Stuckey*, J. Gibbs and G.P. Smith. (SPON: J.A. Sechzer). Dept. Psychiatry, Cornell Univ. Med. Coll., White Plains, NY 10605

Peripheral administration of exogenous bombesin (BBS) produces a large, dose-related inhibition of food intake in rats (Gibbs et al, 1979), suggesting that an endogenous BBS-like peptide plays a role in the physiological regulation of satiety. The site of action and mechanism for the satiety effect of BBS are unknown, but we have shown that the effect does not require the accumulation of ingested food in the gut (Martin & Gibbs, 1980), the abdominal vagus (Smith et al, 1981), or the adrenals (Gibbs et al, 1981). We have now examined whether satiety after BBS requires the pituitary or the sympathetic afferent innervation of the abdominal viscera.

In the first experiment, hypophysectomized male Sprague-Dawley rats (n=11; Hormone Assay, Chicago, IL; 250-300g body weight) were maintained on sweetened Purina mash, 0.15M NaCl and tap water. They were deprived of mash from 1100 to 1400h. Immediately prior to a 30 min access to liquid test diet (25% BioServ, Frenchtown, NJ), BBS or 0.15M NaCl, as the vehicle control, was injected intraperitoneally.

	Dose of BBS ($\mu g \cdot kg^{-1}$)			
	0	2	4	8
Food intake (ml ± SEM)	15.3 ±1.0	13.4 ±1.3	10.6 ±1.2	9.1 ±1.3

BBS inhibited food intake [$F(3,40)=5.48$, $p<.01$] and intakes after the 4 and 8 $\mu g \cdot kg^{-1}$ doses were different from control ($p<.05$). The inhibitions were virtually identical to those we have previously observed in intact rats under the same deprivation conditions.

In the second experiment, 4 male Sprague-Dawley rats underwent spinal cord transection at the sixth thoracic vertebra; 4 unoperated rats served as controls. They were deprived of maintenance food (condensed milk diet) from 1600 to 1000h. Immediately prior to a 30 min access to liquid test diet (40% BioServ), BBS or 0.15M NaCl, as the vehicle control, was injected intraperitoneally.

	Dose of BBS ($\mu g \cdot kg^{-1}$)			
Food intake (ml ± SEM)	0	2	4	8
Cord-sectioned	26.0±2.6	21.2±2.4	16.5±0.3	16.2±2.9
Controls	22.8±2.2	17.2±2.1	16.5±2.6	13.0±2.1

Spinal cord transection did not significantly alter the inhibitory effect of BBS on food intake at any dose.

We conclude that BBS-induced satiety does not require the pituitary or sympathetic afferents from the gut.

This study was supported by USPHS grants AM17240, MH15455, MH00149, and the Irma T. Hirschl Foundation.

255.8 ACQUISITION OF DIETARY PROTEIN/CARBOHYDRATE SELECTION IN NORMAL AND TRIGEMINALLY DEAFFERENTED RATS. M. G. Miller and J. F. Teates*. Food & Drug Administration, Washington, DC, 20204.

Rats given the opportunity to select from diets of different composition choose a stable portion of their daily intake as protein. Although the plasma ratio of tryptophan to neutral amino acids has been implicated as a factor in this control, the mechanisms that mediate between internal metabolic conditions and quantitative diet selection are unclear. The present experiments study the acquisition of the protein selection pattern in normal rats and assess whether oral somatosensory inputs are involved in this process.

Normal rats (mean weight 287g) were offered two isocaloric diets, a protein (soy bean meal, 44% protein) and a carbohydrate (starch) diet, each with equal amounts of fat, vitamins and minerals. At first, the animals ate approximately equal amounts from both diets, resulting in an average intake of 21.0% protein. Over an 8-day period, carbohydrate intake increased, while protein intake decreased until a plateau at 14.7% protein of the daily total intake was reached. Caloric intake was stable throughout the period of observation (4 weeks), and body weight increased at a constant rate. A second group of rats (mean weight 285g) was offered a mixture of equal portions of the two diets used in Expt. 1. After intake and growth rate had stabilized, the rats were subjected either to partial trigeminal deafferentation that selectively eliminated somatosensory afferents from the lower anterior portion of the mouth, or to control surgery. Eight weeks later the deafferented group had recovered control levels of caloric and water intake. (Body weight of deafferented rats remained below control levels due to prolonged hypophagia after trigeminal deafferentation.) At this point both groups were offered the separate protein and carbohydrate diets of Expt. 1. The surgical control group responded similarly to the normal rats of Expt. 1. Within an 8-day period the protein ratio declined from a mean of 21.1% to 12.7%. In contrast, the deafferented group did not acquire stable selection patterns in 5 weeks of observation. Although deafferented rats could distinguish between the two diets, their protein ratio ranged from 0 to 44%, with individual rats fluctuating between both extremes. Unlike the finding in normal rats, the selection challenge eliminated growth in the deafferented group.

We conclude that the quantitative selection of a balanced intake from two diets differing in protein and carbohydrate content requires a considerable time span and is dependent on intact oral somatosensory inputs. We hypothesize that oral somatosensation plays a role in learning quantitative selection of dietary components similar to that which we suggested for the control of total caloric intake in our previous studies.

255.9 DEHYDRATION INDUCED MODIFICATION OF FEEDING AND ITS NEURAL CORRELATE IN THE SLUG, LIMAX MAXIMUS. C.B. Phifer* and D.J. Prior (SPON: L.L. Boyarsky). Physiology Group, Sch. of Biol. Sci., Univ. of Kentucky, Lexington, KY 40506.

The garden slug, Limax maximus, is a terrestrial, moist-skinned animal which lacks a protective shell and is thus extremely susceptible to dessication. Limax displays several behavioral responses which serve to minimize dessication. One of the behaviors modified by the level of body hydration is feeding. Fasted slugs which were dehydrated to 66 ± 4% of their initial body weight (IBW) were less likely to eat dry rat chow than fasted slugs which were kept fully hydrated (i.e. 97 ± 5% IBW; $p<0.0005$, n=21 dehydrated, 20 hydrated).

Data from our laboratory has demonstrated an exponential increase in hemolymph osmolality during progressive dehydration. Based on this relationship, 1.0 molal mannitol was injected into slugs to increase hemolymph osmolality to levels that occur during dehydration. Slugs showed reduced feeding after mannitol injections that were calculated to increase hemolymph osmolality from normal (140 milliosmols/kg H_2O) to that characteristic of a dehydrated animal (70% IBW, 190-200 milliosmols/kg H_2O).

The feeding motor program (FMP) in Limax is a pattern of rhythmic neural activity which can be recorded from an isolated brain and which has been correlated with feeding movements of the buccal mass (Gelperin, A. et al., J. Neurobiol. 9:285, 1978). FMP bouts were initiated in isolated lip-brain preparations by electrical stimulation of lip nerves. Individual FMP bite cycles can be recognized as coordinated bursts of activity from three buccal ganglion roots. The number of bite cycles per FMP bout was reversibly reduced ($\bar{x}$=67% reduction, n=5) when the brain was changed from normal saline to saline of the same concentration as the hemolymph of a dehydrated slug (70% IBW). A similar effect was seen when the brain was bathed in a hyperosmotic (with mannitol) but isoionic saline.

These results suggest that the modification of feeding behavior during dehydration may be mediated by the increase in hemolymph osmolality associated with dehydration. (Supported by a grant from the Whitehall Foundation and by the Graduate School, Univ. of Kentucky.)

255.10 CELIAC VAGOTOMY ALTERS INGESTIVE RESPONSES TO EPINEPHRINE AND HYPERTONIC SALINE BUT NOT INSULIN, 2DG, OR POLYETHYLENE GLYCOL. M. G. Tordoff and D. Novin. Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Evidence for the existence of peripheral sites capable of mediating the regulation of ingestion has come from findings that total subdiaphragmatic vagotomy can attenuate the feeding and drinking responses produced by a number of metabolically or osmotically active compounds. However, the vagus nerve innervates the entire abdominal cavity, making localization of sites within the periphery difficult. In an attempt to localize the site(s) of the peripheral effects, rats with selective celiac vagotomies were exposed to several regulatory challenges.

Groups of 10-12 male Long-Evans rats received celiac vagotomy (C), combined celiac and hepatic vagotomy (CH), low total vagotomy (LT; which destroyed all vagal fibers except gastric innervation), or a sham operation (S). All animals recovered rapidly from surgery, with little (LT group) or no (C and CH groups) postsurgical hypophagia and body weight loss. After a 23-day recovery period, all rats were administered, at 3-4 day intervals, five series of counterbalanced injections inducing metabolic or osmotic regulatory challenges. Insulin (4, 8, 12 U/kg, IP), 2-deoxy-D-glucose (2DG; 125, 250, 500 mg/kg, IP), hypertonic saline (10 ml/kg, 0.25, 0.5, 1.0 M, IP), and polyethylene glycol (10 ml/kg, 30% w/v, SC) were administered in the light period with food and water intake measured 1, 2, 4, and 6 hr later. Additionally, the latencies to eat after 250 mg/kg 2DG and drink after 0.5 M hypertonic saline were recorded. Epinephrine was given at the start of the dark period with food intake measured 30, 60, 90, and 120 min later.

Compared with the S group, the vagotomized groups responded normally after 2DG, insulin, and polyethylene glycol. However, they were less anorexic after epinephrine and drank later and less after hypertonic saline.

Because epinephrine probably acts by stimulating glycogenolysis and activating hepatic metabolic receptors, our results provide evidence that the celiac branch, and not the hepatic branch, of the vagus conveys the majority of hepatic metabolic information to the brain. Our finding that ingestive responses to epinephrine but not 2DG were altered by celiac vagotomy suggests that, in addition to its previously demonstrated action on the liver, 2DG can activate receptors in sites outside the projection field of the celiac vagus. The response to intracellular thirst, according to our results, requires a site that is served by the celiac vagus (eg. the liver or kidneys) and cannot by supported by gastric receptors alone.

255.11 DRINKING IN RESPONSE TO OSMOTIC CHALLENGE IN THE SPONTANEOUSLY HYPERTENSIVE RAT. F.S. Kraly, A.F. Moore*, L.A. Miller*, A. Drexler*, G.A. Holland* and M.A. Meisel*. Psych. Dept., Colgate Univ., Hamilton, NY 13346 and Pharmacol. Sect., Norwich-Eaton Pharm., Norwich, NY 13815.

Adult spontaneously hypertensive rats (SHR) drink more water around mealtime than do normotensive Wistar-Kyoto (WKY) rats when eating solid food at the midpoint of the night phase after 24-hr food deprivation (Kraly et. al., Physiol. Beh., 28, 1982). The mechanisms which account for this nocturnal food-related hyperdipsia in SHR are unknown. Because there is known to be a temporal correlation between the initiation of drinking after a meal and the appearance of increased blood osmolality in the rat, we studied drinking in SHR in response to osmotic challenge produced by loading of salt.

SHR (n=7) and WKY (n=5) rats, determined by tail-cuff to be hypertensive at 10 months of age, had continuous access to milk food and tap water from separate graduated cylinders. NaCl was added to the food such that rats ate milk with 0, .45, .9, 1.8 and 3.6% salt concentration; each concentration of salt was present for 4 consecutive days. Increasing concentrations of salt in food decreased ($p < .001$) 24-hr food intake for both SHR and WKY rats, but SHR ate more ($p < .05$) salted food than did WKY rats. Increasing concentrations of salt in food increased ($p < .025$) 24-hr water intake for both SHR and WKY rats, but SHR drank more water than did WKY rats ($p < .025$). Water to food ratio was the same ($p > .10$), however, for SHR and WKY rats.

Acute cellular dehydration was produced by i.p. 1M NaCl (.06, .12, .25, .5 and 1.0% body weight) at the midpoint of the night phase in 10 month old SHR (n=10) and WKY (n=6) rats maintained on standard solid chow and water: Threshold dose for increased drinking above baseline was .25% for WKY but .12% for SHR. SHR drank more ($p < .005$) in a 1-hr test than did WKY rats across the range of doses. Cellular dehydration was not differentially effective, however, for eliciting drinking in the night phase vs. the day phase: For example, while SHR drank more after .5% 1M NaCl than did WKY both in the day ($p < .01$) and night ($p < .05$) phases, cellular dehydration failed ($p > .10$) to provoke more drinking at night than in the day in SHR and WKY rats.

Thus, nocturnal food-related hyperdipsia in SHR does not appear to be fully explained simply by hyperdipsia in response to osmotic challenge.

256.1 EFFECT OF HYPERTONIC NaCl APPLIED TO THE LATERAL PREOPTIC AREA ON WATER INTAKE AND ON THE HEMODYNAMIC ALTERATIONS CAUSED BY INTRAVENOUS INFUSION OF ANGIOTENSIN II. W.A. Saad, L.A.A. Camargo*, J. V. Menani*, W.A.Saad* and A. Renzi*. Department of Physiology and Pathology, school of Dentistry - UNESP - Araraquara, 14.800, S.P. Brasil, and school of Medicine - USP - São Paulo - SP.

Hyperosmolarity and angiotensin II (AII) induce thirst and hemodynamic alterations by acting directly on the brain (Anderson,B.,Acta Physiol.Scand.28,1953; Epstein et al.,J.Physiol. (Lond.) 210,1970). Rats submitted to lesion of the anteroventral third ventricle (AV3V) become chronically hyperosmotic and hypernatremic after recovery and show decreased water intake and pressor and antidiuretic responses after injection of hypertonic saline or angiotensin II (AII).The main objective of the present study was to investigate whether the effects of application of hypertonic NaCl to the lateral preoptic area (LPO)would modify water intake and the hemodynamic alterations caused by systemic application of AII,that might have these effects by acting on periventricular structures.Rats bearing cannulae in the LPO were implanted with a catheter in the jugular vein and in the femoral artery,which permitted recording arterial pressure and heart rate, as well as water intake in unesthetized animals.Intravenous infusion (i.v.i)of 10-40ng AII caused a dose-dependent increase in water intake and arterial pressure.

I.v.i. of 40ng/ul/min AII increased water intake (3.65 $\pm$ 0.28 ml),and application of 0.2M NaCl to the LPO also increased water intake (2.41 $\pm$ 0.25 ml) to a total of 5.87 ml. I.v.i. of AII caused a 47mmHg increase in blood pressure,whereas application of NaCl to the LPO had no effect on arterial pressure.After AII infusion, heart rate decreased 33bpm,whereas application of NaCl to the LPO not changed these values.A parallel study was done in animals with cannulae implanted in both LPO and subfornical organ (SFO).Application of 0.2M NaCl into the LPO increased water intake (1.73$\pm$0.19ml)and the injection of AII into the SFO also increased the water ingestion (3.32$\pm$0.65ml). Blood pressure also increased after injection of NaCl 0.2M NaCl into LPO (21mmHg),the AII injection to the SFO produced higher increase (32mmHg).Heart rate increased 22bpm after application of 0.2M NaCl to the LPO whereas the injection of AII into the SFO decreased 25bpm in relation to the values of the application of NaCl to the LPO.Thus, we can conclude that there is interaction between the LPO,which is Known to be an osmosensitive region,and other areas that are receptive to changes in osmolarity and to the effect of AII,such as the periventricular structures.Both may interact in the maintenance of the fluid and electrolytic balance with systems that regulate arterial pressure.

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256.2 INTRACEREBROVENTRICULAR ANGIOTENSIN III IS DIPSOGENIC IN RATS AND GERBILS. J. W. Wright, S.L. Morseth*, M.J. Mana*, E. LaCrosse* and J. W. Harding. Departments of Psychology and Veterinary Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164.

Previous research has established that the octapeptide angiotensin II (AII) is dipsogenic when administered intracranially or intracerebroventricularly (icv) in many species (for review see Fitzsimons, Fed. Proc., 37:2669, 1978). The location of central receptors responsible for monitoring the elevated levels of AII in the cerebrospinal fluid is vigorously debated, however circumventricular organs (CVOs) are frequently offered as candidates. Our laboratory measured the ^{125}I-AII binding capacity of several brain regions including CVOs in rats, hamsters, kangaroo rats, gerbils, degus and mice. The results indicated significant binding in CVOs, and many other regions in rats and mice but a surprising lack of binding in any brain region of gerbils and degus (Harding, Stone and Wright, Brain Research, 205: 265, 1981). When this experiment was repeated with ^{125}I-AIII considerable binding was noted in many brain regions of rats and gerbils (see accompanying abstract, Harding, Petersen and Wright). On the bases of these results icv injections of AII and AIII (doses 0.01, 0.1, 1 and 10 ng) were administered to rats, which possess CVO binding capacity for both AII and AIII, and to gerbils which show CVO binding only for AIII. The results indicate that rats drank slightly more water during a post-injection 30 min period to AIII than to AII. Gerbils also appeared to drink more water to icv AIII than AII.

These results suggest that dipsogenicity to icv angiotensin II in gerbils may occur due to its in vivo conversion to the heptapeptide form, or a fragment, that activates AIII receptors.

256.3 THE INITIAL CHARACTERIZATION OF "APPARENT" ANGIOTENSIN III BINDING SITES IN RODENT AND PRIMATE BRAINS. J. W. Harding, E. P. Petersen and J. W. Wright. Departments of Veterinary Comparative Anatomy, Pharmacology and Physiology, and Psychology, Washington State University, Pullman, WA 99164.

The majority of previous studies on the interaction of angiotensins with the CNS have focused on the role of the octapeptide, angiotensin II (AII). It is established that AII has potent pressor and dipsogenic activities on most every species examined. However, many of these species exhibit a paucity of ^{125}I-AII binding to CNS membrane preparations. Two explanations seem possible to account for this apparent paradox: 1) The assay procedures being utilized in several laboratories are inappropriate for detecting AII binding in many species. 2) Or, AII is not the actual ligand that interacts with the receptor site. In this abstract we describe the existence of apparent angiotensin III (AIII) binding sites in gerbil brain. This is of interest since intracerebroventricular AII injections induce drinking in gerbils, a species that has little capacity to bind ^{125}I-AII to brain membrane preparations. These binding sites are widely distributed throughout the brain. The binding is 80% reversible with a $t_{\frac{1}{2}}$ of association of 10 min at 37^0 C. The Kd's for several angiotensin-like peptides in gerbil are as follows: des-Asp AI - 620nM, Ile - AIII - 1μM, AIII - 1.1 μM, AI - 3.7μM, AII - 11 μM, Sar^1, Leu^8-AII - 60 μM, and Sar^1, Ala^8-AII - 100 μM. The relatively high Kd values which have been determined are most likely manifestations of extensive degradation. Using radioiodinated angiotensins and thin layer chromatography it is possible to demonstrate that over 99% of ^{125}I-AIII is degraded during incubation. Under the same conditions ^{125}I-AII is less than 10% degraded. This result suggests that we may be overestimating the concentration of displacers and Kds by 100 fold or more. Of additional interest is the fact that the ^{125}I-ligand which is bound to the receptor is neither AII nor AIII and appears to be a specific metabolite of AIII. Preliminary studies in our laboratory show high concentrations of similar receptors in other rodent and primate species.

256.4 THE SUBFORNICAL ORGAN-MEDIAN PREOPTIC PATHWAY: EVIDENCE SUGGESTING THAT AN AII-LIKE SUBSTANCE MAY BE A NEUROTRANSMITTER IN THIS PROJECTION. T.S. Gray, M.D. Cassell, T.H. Williams, R.W. Lind and A.K. Johnson. Depts. of Anatomy and Psychology, Univ. of Iowa, Iowa City, IA 52242.

Angiotensin II (AII) elicits drinking, and pressor responses and vasopressin release. One of the possible central sites of AII action is the subfornical organ (SFO). Over the past few years, evidence, although controversial, has accumulated suggesting that AII is a neurotransmitter and/or neuromodulator in the brain. AII-like immunoreactivity has been localized in variable quantities in many regions. Thus, like other peptides, AII may have both peripheral hormonal and central neurotransmitter functions.

In summarizing the data implicating AII as a mediator of thirst, we recently proposed that neural information from AII receptors in the SFO is relayed to cells in the ventral median preoptic nucleus (MPO). Supporting evidence comes from studies that have demonstrated that selective destruction of this nucleus blocks drinking responses to both peripheral and central AII, while SFO lesions block drinking to peripheral injections only. It has been suggested that AII may serve as a neurotransmitter in this SFO-MPO projection.

As a first step towards testing this hypothesis, we have compared sections from brains with SFO HRP injections which labeled the fibers of the SFO-MPO pathway and homologous brain sections processed for AII-like immunoreactivity using the Sternberger PAP technique. A high degree of correspondence was observed between the HRP labeled fibers of the SFO-MPO pathway and AII-immunoreactive fibers. This included observations of fibers which extended down the midline and continued ventrally rostral to the anterior commissure to enter the MPO. HRP-reactive and AII-immunoreactive fibers both appeared to terminate in within the median preoptic nucleus.

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256.5 LATERAL PARABRACHIAL NUCLEUS LESIONS ELICIT AN EXAGGERATED DRINKING RESPONSE TO ANGIOTENSIN II AND HYPERTONIC SALINE. L. E. Ohman* and A. K. Johnson (SPON: J. Stein). Department of Psychology, University of Iowa, Iowa City, IA 52242.

Changes in blood pressure and volume are known to activate mechanisms controlling body fluid balance (e.g., drinking). Recent anatomical investigations have revealed major ascending projections from the nucleus tractus solitarius to the parabrachial nucleus (PBN) (Loewy and Burton, J Comp Neurol, 181:421-450, 1978; King, J Comp Neurol, 191:615-638, 1980) and in turn, heavy projections from the lateral PBN (LPBN) to the hypothalamus, particularly the median preoptic nucleus, a prominent component of the AV3V region (Saper and Loewy, Brain Research, 197:291-317, 1980). Given that the NTS receives numerous inputs from neurons carrying cardiovascular information, and that the AV3V plays an important role in maintenance of fluid balance, it might be hypothesized that pressure/volume related information is carried in a pathway from the NTS to the AV3V via the PBN.

As a first test of this hypothesis the importance of the LPBN in the response to drinking challenges was examined. Rats with bilateral LPBN, sham lesions, frontal cortex lesions, and intact controls were tested for drinking in response to subcutaneous administration of AII (3 mg/ml and 1.5 mg/ml), isoproterenol (100 µg/kg and 30 µg/kg), hypertonic saline (12% and 4%) and isotonic saline. PBL lesion animals drank significantly more than controls to AII (3 mg/ml $p < 0.001$, 1.5 mg/ml $p < 0.001$) and to hypertonic saline (12% $p < 0.001$, 4% $p < 0.01$) but no difference was found between groups for isoproterenol (100 µg/kg $p < 0.13$, 30 µg/kg $p < 0.94$) or isotonic saline ($p < 0.73$).

In a second experiment intact and nephrectomized rats were administered polyethylene glycol. No significant difference in drinking response to extracellular depletion was found between lesion and control groups.

The results from these experiments show that rats with LPBN lesions overrespond to challenges that result in elevation of blood pressure (i.e., AII and hypertonic saline) but do not differ from controls in response to challenges that reduce pressure. These data suggest that the LPBN may send inhibitory signals to the hypothalamus in response to increases in blood pressure and that destruction of the nucleus removes inhibition leading to overresponding. The LPBN appears to play no role in the mediation of thirst related to low blood pressure or volume information.

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256.6 ANGIOTENSIN OF CEREBRAL ORIGIN IS THE OPTIMAL SYNERGIST WITH DOCA FOR THE AROUSAL OF A SODIUM APPETITE. A.N. Epstein and E.C. Jameson*. Dept. of Biology, Univ. of Pennsylvania, Phila., PA 19104.

We have proposed (Fluharty, et al, Neurosci. Abstracts, 6: 34) that a synergy of angiotensin and mineralocorticoid is the means by which the brain is apprised of the need for salt in the sodium deficient animal. Here we compare the effectiveness, for this synergy, of angiotensin that is generated in the brain with that which is blood-borne to the brain in imitation of angiotensin of renal origin. Renin (mouse submaxillary, generous gift of T. Inagami) was used because of the potency and long duration of its angiotensinogenic action. In a preliminary experiment, the synergy phenomenon was confirmed. Rats bearing Silastic capsules (N=11) delivering DOCA (~300 µg, sc, for 6 days) the precursor of aldosterone, and receiving renin for the first time by intracerebroventricular (ICV) injection (2 or 3 ng) drank salt (3% NaCl in water) more consistently (10/11 vs. 4/8), sooner (6.3' vs. 21.8', nondrinkers not included), in greater volume (3 hr post-renin: 9.5 vs. 2.4 ml; 18 hr post-renin: 31.7 vs. 4.0 ml), and with more persistence (9.6 vs. 1.0 ml at 72 hr post-renin) than rats that received the same ICV renin without prior steroid treatment (N=8). The effects of intracerebral renin were then compared with those of intravenous renin. All animals were treated with systemic DOCA. ICV renin (same data as above) was more effective, especially for rapidity and persistence of salt intake, than was intravenous (N=16). Giving the data for ICV vs. IV: latencies of drinkers were 6.3' vs. 27.7', and post-renin intakes were: 9.5 vs. 7.4 at 3 hr, 31.7 vs. 15.5 at 18 hr, 9.6 vs. 6.6 at 72 hr. Lastly, the evidence for synergy was sought when the renin was given only intravenously. Animals treated with DOCA (N=16) and those that were not (N=12) received 5 or 6 µg of renin IV. Their latencies to drink salt did not differ nor did their intakes at 3 hr post-renin (7.4 vs. 7.7 ml); 18 hr later the DOCA R_x animals had drunk somewhat more salt (15.5 vs. 7.6).

Thus, 1) intravenous and intracerebroventricular renin without DOCA produce some salt intake, 2) the synergy of the two hormones for the production of a salt appetite is inconspicuous when renin is given intravenously, but 3) strong salt solution is drunk reliably, rapidly, in a large volume, and persistently when intracerebral renin synergizes with DOCA. Angiotensin of cerebral origin may be the natural synergist of aldosterone in arousing the behavioral defense against sodium deficiency. Supported by NS 03469.

256.7 THE NECESSITY OF ENDOGENOUS ANGIOTENSIN FOR SODIUM APPETITE IN THE SODIUM-DEPRIVED RAT. Karen Moe*, Mark Weiss*, and Alan Epstein (SPON: Eliot Stellar). Dept. of Biology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Rats (N=6) were trained to drink salt by restricting them to sodium-deficient diet for 7 days and giving them a daily 2-hr access to 3% NaCl (in water) in addition to water and the diet. On Day 8, a salt appetite was provoked with furosemide (10 mg/ml/rat, subcutaneous), a diuretic and natriuretic and a potent releaser of renin, given 2 hr before the daily 2-hr presentation of salt. The appetite was attenuated in all animals (mean percent decrease = 81%) by concurrent intravenous administration of Captopril (Squibb), a competitive inhibitor of the enzyme that converts angiotensin I to angiotensin II (A II). The Captopril was infused at 0.42 mg/min beginning 30 min before the furosemide and continuing through the salt access period for a total of 270 min, thereby delivering a total dose of 112.5 mg/rat. Rats infused with the Captopril vehicle (0.9% NaCl) in conjunction with the furosemide drank an average of 6.7 ± 0.3 ml of 3% NaCl and 6.0 ± 2.0 ml of water during the 2-hr salt access period, whereas rats that received Captopril with the furosemide consumed an average of 1.4 ± 0.8 ml of saline and 1.8 ± 1.2 ml of water. Thus, Captopril treatment blocked both the salt and water consumption elicited by the furosemide.

A second experiment showed that rats (N=3) remained behaviorally competent during the combined furosemide-Captopril treatment. They drank promptly and copiously to a pulse intracerebroventricular injection (pICV) of angiotensin II (3 ng; 11.3 ± 2.5 ml average water intake in 30 min), while receiving the same Captopril treatment as in the previous experiment. But they drank less or not at all to pICV angiotensin I (3 ng), confirming the effectiveness of the AI→AII blockade in the brain. (Water intake in response to pICV A I plus IV infusion of vehicle was 10.7 ± 1.7 ml, compared to 0.3 ± 0.3 ml consumed when the pICV A I was combined with IV Captopril infusion.)

We have proposed that a synergy of mineralocorticoid and angiotensin apprises the brain of the need for salt and is necessary for arousal of a sodium appetite. The present results, showing the suppression of the appetite when rats cannot generate angiotensin II, is compatible with this idea. Moreover, these results suggest that in an animal whose angiotensin-aldosterone system has been activated by sodium deprivation, a sudden rise in endogenous angiotensin may be a necessary trigger for the arousal of a salt appetite.

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256.8 THE EFFECT OF PERIPHERALLY AND CENTRALLY ADMINISTERED PROLACTIN (PRL) ON THE RESPONSES TO SOME DIPSOGENIC STIMULI. S. Kaufman* and M.D. Evered* (SPON: Dr. K.G. Pearson) University of Alberta, Edmonton, Alberta T6G 2G3

Chronic hyperprolactinaemia, produced by implanting ectopic pituitary glands, has recently been shown to be associated with an increase in the spontaneous 24 hr water intake of the rat (Kaufman et al. 1981). In the present study, the drinking response of similarly prepared rats to IV infused angiotensin II (AII, 0.2 µg/min for 30 min) was shown to be greater (2.4±0.7 ml, n=7) than that of the normoprolactinaemic controls (1.0±0.4 ml, n=8, $p < 0.05$) whereas their response to IV hypertonic saline (5ml 2M-NaCl/kg body wt.) was attenuated for the first hour of drinking (hyperprolactinaemic 24.2±3.3 ml/kg body wt., n=7; controls 32.9±2.3 ml/kg body wt., n=10, $p < 0.01$). After 48 hr IV infusion of PRL (64 µg/hr), but not after 3 hr, the drinking response to intracerebroventricular AII (10pM) was increased (mean increase 1.9±0.3 ml, n=8, $p < 0.005$) whereas the response to intracerebroventricular carbachol (300 ng) was attenuated (mean decrease 3.3 ±1.0 ml, n=6, $p < 0.001$). However, when PRL was infused into the lateral ventricle (1 µg/hr for 48 hr) the drinking response to centrally administered AII (10 pM) was attenuated (mean decrease 2.9±0.9 ml, n=9, $p < 0.01$). Neither central nor peripheral infusions of PRL modified the water intake of 24 hr water deprived rats.

It is concluded that peripheral hyperprolactinaemia increases the drinking response to the 'extracellular' stimulus of AII whereas the responses to the 'intracellular' stimuli of NaCl and carbachol are attenuated. However, PRL acts centrally to attenuate the response to AII. These results are consistent with the concept that PRL interferes with the vasoactivity of AII.

Ref. Kaufman, Mackay & Scott, J. Physiol. 321 : 11-19 (1981).

257.1 HORMONAL ACCELERATION OF FAT DEPOSITION AND SECONDARY HYPERPHAGIA IN RATS. L. D. Devenport, C. G. Murray*, and A. Torres*. Department of Psychology, University of Oklahoma, Norman, Oklahoma 73019

The administration of deoxycorticosterone (DOC, 3 mg/kg daily) or aldosterone (Aldo, 0.25 mg/kg daily) against a background of corticosterone replacement (Cort, 3 mg/kg daily) in adrenalectomized rats significantly augmented daily food intake (lab chow) and elevated body weight relative to adrenalectomized animals receiving only Cort. As wet/dry tissue analysis and hematocrit determination disclosed no differential fluid retention among groups and in view of equivalent body lengths, the change in body weight was attributed to adipose tissue. Axillary fat-pad weights were significantly heavier in the DOC and Aldo groups, supporting this interpretation.

Other groups of animals were treated identically except that they were restricted to a fixed amount of lab chow set at about 75% of normal intake. Spillage was collected and refed periodically in the form of fresh chow along with the daily allotment. On this regimen, Cort-only rats progressively lost weight. Remarkably, DOC and Aldo rats gained weight under these starvation conditions. Again, no differences in body length or fluid retention were found.

The results suggest that DOC and Aldo enhance the diversion of caloric intake to fat deposition at the expense of other cells. We say this because of the increased intake of free-fed Aldo and DOC animals. A change in general metabolic rate could account for the weight differences of restricted rats but it would not predict the relative hyperphagia under ad lib conditions. Rather, a compensatory hypophagia might have been expected. This was not the case. Whether this caloric diversion was a direct result of mineralocorticoid action we cannot say. There is reason to believe that Aldo may enter into a loose regulatory relationship with β-lipotropin (Matsuoka et al. Science, 1980, 209, 307-308), which in turn may be directly responsible for the effects we observed. Likewise, we are unsure as to whether the hormonal action--whether peptide or steroid--is acting directly on fat deposition or on a mechanism of energy expenditure (e.g. thermogenesis).

257.2 EFFECTS OF LESIONS OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS ON PLASMA INSULIN LEVELS IN THE RAT. J. P. Steves* and J. F. Lorden (SPON: J. W. Brown). Dept. of Psychology, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The paraventricular nucleus of the hypothalamus (PVN) has been implicated in the control of feeding in the rat. This region has been shown to be the area most sensitive to adrenergically stimulated feeding (Leibowitz, Pharmacol. Biochem. Behav., 8: 163-75, 1978) and lesions in this area produce obesity. Gold et al. (Physiol. Behav., 18: 1111-19, 1977) have suggested that destruction of the connections of the PVN is the basis for the obesity syndrome seen after large lesions aimed at the ventromedial hypothalamus (VMH). Since recent studies by Cox and Powley (Am. J. Physiol., 3: 566-83, 1981) have demonstrated the critical importance of hyperinsulinemia in the VMH obesity syndrome, it would be necessary to demonstrate that damage to the PVN is capable of producing hyperinsulinemia in order to argue that the PVN plays an important role in the VMH syndrome. Anatomically, the PVN is connected to brainstem autonomic nuclei, suggesting that damage to the PVN might alter vagal control of the pancreas. Thus, we have attempted to pursue the relationship between the PVN and VMH syndrome by comparing the effects of PVN and VMH lesions on the production of hyperinsulinemia. Chronic Silastic catheters were implanted in the inferior vena cava of adult female rats on Day 1 of the experiment. On Day 3 a series of blood samples was taken after a fast of at least 5 and no more than 10 hours. Immediately after a baseline sample was drawn, .3 cc of 2 M glucose was injected through the catheter. Two additional blood samples were taken 5 and 15 min later. On Day 5, the animals were given an electrolytic lesion (1 mA, 10 sec) aimed at either the VMH or PVN. On Day 6, animals were again fasted for 5 hours and the blood sampling procedure was repeated. Following the postsurgical blood sampling, animals were maintained on a high fat diet. Body weights were recorded every 5 days until sacrifice 1 month later. Blood samples were assayed for glucose and insulin. Both VMH and PVN lesions produced an increase in glucose-stimulated insulin levels in comparison with the preoperative values and prior to the onset of obesity. Body weight data obtained from animals with histologically verified lesions indicated similar weight gains in both groups. Examination of the lesion sites also indicated that the PVN lesions spared the ventromedial nucleus. While we have not yet shown that the VMH syndrome is the result of damage to fiber connections of the PVN, the finding that PVN lesions are capable of producing an immediate hyperinsulinemia adds strength to the argument. (Supported by NINCDS grant NS 14755.)

257.3

WITHDRAWN

257.4 NOREPINEPHRINE- AND d-ALA-MET-ENKEPHALINAMIDE-ELICITED FEEDING FROM THE PVN CAN BE DISSOCIATED BY DEXAMETHASONE INJECTION OR ADRENALECTOMY. S. McLean* and B. G. Hoebel. Dept. Psychology, Princeton Univ., Princeton, NJ 08544

Infusion of either norepinephrine (NE) or d-ala-met-enkephalinamide (DALA) into the paraventricular nucleus of the hypothalamus (PVN) elicits feeding; microinjection of an alpha-adrenergic receptor blocker attenuates opiate-elicited feeding. This suggests the opiates might act through the local release of NE. If so, manipulations that affect NE elicited feeding should have a similar effect on DALA-elicited feeding. However, we find these two effects can be dissociated by manipulation of the pituitary-adrenal axis.

Six groups of 6 male Sprague-Dawley rats (350-400 g) with cannulas in PVN received saline or dexamethasone (DEX) injected s.c. at doses of 400 ug/kg 24 hrs and 200 ug/kg 2 hrs before DALA (4 ug), NE (12.8 ug) or saline infusion into the PVN.

The results showed that DEX potentiated feeding induced by NE but not DALA.

I.P. Injection	SAL	DEX	DEX	SAL	SAL	DEX
	+	+	+	+	+	+
PVN Injection	DALA	DALA	SAL	SAL	NE	NE
Food Intake (gm)	1.7	1.6	0.2	0.1	2.6	4.7

To determine whether adrenalectomy has the opposite effect of DEX, 15 animals that increased their food intake in response to both NE and DALA received a bilateral adrenalectomy, and 14 received a sham operation. Eight days later both groups were injected with NE, DALA, or saline in counter balanced order on separate days.

	SAL	DALA	NE
Baseline (gm)	0.4	2.6	3.5
Sham (gm)	1.5	2.3	3.1
Adrenal. (gm)	0.7	2.6	1.9*

*Adrenalectomy reduced NE-induced feeding relative to sham ($p<.01$).

In summary, NE-elicited feeding was potentiated by injection of dexamethasone and reduced by adrenalectomy. In contrast, DALA-induced feeding was unaffected by either manipulation. This dissociation suggests that opiate-induced feeding following injection into the PVN is not dependent on the glucocorticoid-sensitive NE feeding circuitry.

257.5 EXPERIMENTAL DIABETES IN RATS: MACRONUTRIENT SELF-SELECTION AND BRAIN ^{3}H-SPIROPERIDOL BINDING. T.J.Bartness* and N.Rowland* (SPON: C. VanHartesveldt). Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

The self-selection among three pure macronutrient sources (carbohydrate, CHO, protein and fat) differed between diabetic and non-diabetic control rats. There were further differences between Sprague-Dawley (SD) and Long-Evans (LE) rats, and among each of four different types of experimental diabetes (Table).

Group	Treatment	K*	% calories selected		
			CHO	Protein	Fat
1 (SD)	Control	3.1	47	37	16
2 (SD)	70 mg/kg SZ, iv	0.5	19	59	22
3 (SD)	45 mg/kg SZ, iv	1.2	31	38	31
4 (SD)	Pancreatectomy	1.2	23	38	39
5 (LE)	Control	3.5	16	55	29
6 (LE)	90 mg/kg SZ, ip at 2 days of age	1.0	8	44	48

(SZ=streptozotocin; K= Conard glucose tolerance coeff. Mean x10^2)

High protein intakes were observed in all diabetic groups, but increased fat intake was not observed in the most severely diabetic animals, as indicated by iv glucose tolerance test (Table), and by elevated basal levels of glucose, FFA, ketones.

In a second phase of the experiment, daily treatment with replacement doses of insulin reversed the plasma metabolite levels and the diet selection profiles toward normal.

In a third experiment, it was demonstrated that the increased protein intakes are indeed regulated. Intragastric preloads of protein, but not of CHO or fat, selectively suppressed the self-selection of protein in all diabetic groups.

The increased protein intake may have neurochemical consequences, since elevated protein intakes restore the decreased amphetamine sensitivity of diabetic rats toward normal (Marshall, 1978). Diabetic rats from the above groups, now feeding chow, were sacrificed and the relative number of ^{3}H-spiroperidol binding sites determined in homogenates of striatum and frontal cortex. No systematic differences were found between the diabetic and control groups in dopamine D2 receptor binding in striatum (using various ligand concentrations, and (+) butaclamol for nonspecific binding); these data do not confirm the report by Lozovsky et al (1981). We further found no difference in the dopamine (butaclamol-displacable binding) or serotonin S2 (cinanserin-displacable binding) in the frontal cortex.

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257.6 DOPAMINERGIC UNIT ACTIVITY IN FREELY MOVING CATS: LACK OF CORRELATION WITH FEEDING AND GLUCOSE INJECTIONS. Robert E. Strecker, George F. Steinfels, James Heym and Barry L. Jacobs. Prog. in Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

We have previously reported that the activity of dopaminergic (DA) neurons in the substantia nigra (SN) of freely moving cats is characterized by constant discharge rate and pattern across the sleep-wake cycle (Life Sci. 29:1435, 1981) and is also responsive to environmental stimuli (Steinfels et al., this volume). The recording technique that we utilize has enabled us to obtain neuronal correlates of physiological functions in which the mesencephalic dopamine system is thought to play a role. Since brain dopamine has been hypothesized to play a role in the mediation of feeding behavior we have examined DA unit activity in the SN of freely moving cats during feeding, satiety, and after systemic injections of glucose. Cats were food deprived for 24 hours and then baseline single unit activity was recorded by means of a movable microwire technique (Life Sci. 29:1435, 1981). Unit activity was monitored throughout feeding and for 90 minutes post-prandially. Relative to baseline, there was no significant variation in unit activity at any time during feeding or in the post-prandial satiety period. These data are surprising considering the accumulation of data from drug and lesion studies that implicate the nigrostriatal dopamine system in the control of food intake. Furthermore, a previous electrophysiological study in the anesthetized rat found a 60 to 100 percent decrease in DA unit activity in the SN after i.v. or s.c. injections of glucose (Saller and Chiodo, Science 210:1269, 1980). However, we observed no change in unit activity following i.p. injections of glucose (300, 500 and 1000 mg/kg) or glucose in combination with insulin (300 mg/kg glucose and 0.8 units/kg insulin). Possible causes of the discrepancy between these two studies of DA unit response to glucose injections are currently being examined (species differences, anesthesia, route of administration). Despite these continuing unresolved issues regarding the effect of glucose, one clearcut conclusion from our data should be kept in mind: the activity of SN DA neurons in the freely moving cat is not changed during feeding or by satiation produced by food consumption. In conclusion, the lack of variability of DA unit activity during feeding and satiety, and after glucose injections, does not support the hypothesis that the nigrostriatal dopaminergic system plays a role in the mediation of feeding behavior. (Supported by NSF BNS 81-19840, DA 05224-01 and the American Philosophical Society).

257.7 EFFECTS OF A QUATERNARY OPIATE AGONIST ON FEEDING AND BODY TEMPERATURE. F. S. Tepperman, M. Hirst* and S. Tse*. Department of Pharmacology and Toxicology, The University of Western Ontario, London, Ontario, Canada.

Opiate receptors are located throughout the gastrointestinal tract and brain in tissue associated with ingestive processes. We have previously demonstrated feeding following central injections of morphine sulfate and have begun investigation of a quaternary derivative, morphine methiodide. Its charged cationic structure would tend to deter it from moving across lipid membranes and so it would be much less likely to migrate from its injection site in the brain (or cross the blood brain barrier). Because of its altered structure, we prepared for the feeding and temperature studies by establishing that 1) morphine methiodide has opiate agonist activity since it could inhibit electrically evoked twitches in the guinea-pig ileum while codeine methiodide, a congener with similar charge and size had no activity. Morphine methiodide was about 35 times less potent in this regard than morphine; 2) when given i.v. to rats, morphine sulfate had a small but prolonged effect on blood pressure, while morphine methiodide caused a greater fall but one of shorter duration.

For the feeding studies, male Sprague-Dawley rats were housed individually and maintained on a 12-hour light-dark cycle. All were implanted stereotaxically with a cannula which extended to the right ventromedial hypothalamus (VMH). Food eaten and core temperature were recorded hourly for 3 hours during the light period. Studies included: 1) morphine (5.3 nmole), morphine methiodide (10.6 nmole and 21.2 nmole) and saline were instilled into the VMH in a Latin square design. Morphine and morphine methiodide (21.2 nmole) stimulated an equivalent amount of food intake which was significantly greater than that following saline, but the methiodide caused a much smaller degree of hyperthermia than did the base, suggesting that the latter had migrated much more effectively than the former to temperature-sensitive sites; 2) morphine methiodide and codeine methiodide (21.2 nmole) were instilled into the VMH in a cross-over design. Morphine methiodide stimulated significantly more feeding than the codeine congener but there were no differences in temperature; 3) naloxone was instilled into the VMH (10.6 nmole) or given s.c. (2 and 10 mg/kg) or saline was given by the same routes just prior to instillation of morphine methiodide (21.2 nmole) into the VMH. When given centrally, naloxone did not affect the appetitive stimulation induced by morphine methiodide, but s.c. naloxone caused a dose-related depression in feeding. Core temperature was not affected by naloxone administered by either route.

(Supported by the Medical Research Council of Canada.)

257.8 THE EFFECTS OF GUANETHIDINE SYMPATHECTOMY ON VENTROMEDIAL HYPOTHALAMIC OBESITY. M. Walgren*, W. Laughton*, and T.L. Powley. Laboratory of Regulatory Psychobiology, Purdue University, West Lafayette, IN 47907.

Animals with ventromedial hypothalamic (VMH) lesions exhibit both increases in parasympathetic responses and decreases in sympathetic tone. Two experiments were performed to determine if decreases in sympathetic tone alone were sufficient to either (1) produce the essential elements of the VMH syndrome or (2) potentiate the expression of the syndrome.

In the first experiment, 5 weeks of guanethidine (GUAN) administration (40mg/kg/day) to male Sprague Dawley rats produced a relatively complete sympathectomy (SYMPX) as measured by cell loss in the superior cervical ganglion ($\bar{X}$= 81% depletion) and decreased salivary gland weight ($\bar{X}$= 13% reduction). GUAN-treated animals did not display any characteristics of the VMH syndrome and, in fact, evidenced a mild but significant depression in both body weight and food intake. Maintenance of the animals on a palatable high-fat diet did not unmask any subtle counterpart of the VMH syndrome in the SYMPX group. Subsequent production of VMH lesions (electrolytic, 1ma for 18sec) in SYMPX and saline-treated animals revealed no potentiation of the VMH syndrome by the prior GUAN treatment. In addition, determination of free fatty acid (FFA) mobilization responses of the animals indicated that VMH lesions elevated basal FFA levels but did not impair the mobilization of FFAs to a 2DG challenge. These results indicate that the classical obesity, hyperphagia, and finickiness of the syndrome can be expressed without the impairment in FFA mobilization that has been reported to follow some basomedial hypothalamic lesions.

The second experiment employed a longer treatment of GUAN (40 mg/kg/day for 5 weeks, 50 mg/kg/day for a sixth) and eliminated adrenal catecholamines as a factor by prior demeduallation (DEMED). SYMPX ($\bar{X}$= 85% depletion of superior cervical ganglion), with or without prior DEMED, failed to produce any of the elements of the VMH syndrome. The subsequent introduction of lesions and an extended (16 week) high-fat feeding period did reveal that the SYMPX could potentiate (13% increase) the effect of VMH lesions on body weight.

Taken together, these results do not support the conclusion that a preponderance of the VMH syndrome can be explained by a simple reduction in sympathetic tone of the sort produced by GUAN. The results do indicate, however, that a reduction in sympathetic activity can facilitate, to some extent, the weight gain that VMH lesions produce.

258.1 REVERSAL VOLTAGE OF CURRENTS INDUCED BY PRESSURE-INJECTION OF cGMP INTO SOLITARY RODS. L. H. Pinto and J. E. Brown*. Dept. of Biological Sciences, Purdue University, W. Lafayette, IN 47907

Solitary rods from the retinas of large neotenic Ambystoma tigrinum prepared by the method of Werblin (J. Physiol. 280:449, 1978) were impaled with double-barreled electrodes. One barrel (filled with 1.0 M K-acetate) was used to record membrane voltage; the second barrel (filled with 250 mM K-acetate, 10 mM cGMP) was used to pass voltage-clamp current and to pressure inject cGMP. The two barrels had less than 10 M ohm coupling resistance and pressure-ejection of cGMP solution into the bath produced no significant voltage-clamp current. At normal, dark resting voltage, pressure-injection of cGMP into rod outer segments (10 cells) induced inward current, both in control Ringer's (103 mM NaCl, 2.5 mM KCl, 1.3 mM $MgCl_2$, 3.4 mM $CaCl_2$, 2.8mM HEPES, pH 7.8) and in solution in which the Na^+ was replaced by choline$^+$ (one cell). The pressure-injection of 250 mM K-acetate did not induce changes of membrane current either at normal, dark resting voltage or at voltages up to 40 mV positive to resting voltage. In control Ringer's solution, the cGMP-induced current reversed sign at voltages between 30-45 mM positive to normal, dark resting voltage (for 5 cells). Light-induced current was not reversed in sign for any of the 5 cells at these voltages.

We conclude that the cGMP-induced change in conductance of the rod membrane is different from the light-induced change in conductance.

258.2 CONTROL OF THE LIGHT-SENSITIVE CURRENT OF ROD PHOTORECEPTORS BY cGMP AND EGTA. Stuart A. Lipton* (SPON: T.N Wiesel). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Both cGMP and calcium have been proposed as internal messengers in retinal rod outer segments. Internal messengers are believed to mediate the response and adaptation to illumination by modulating an inward, "light-sensitive" current. Since several experiments have suggested that the levels of cGMP and calcium change with photostimulation, it was of interest to determine if these substances affect the light-sensitive current. Thus solitary, cultured rods were voltage-clamped and treated with TEA, cesium, and cobalt to isolate the light-sensitive current (Bader, C.R. et al., Neurosci. absts. 7, 728, 1981).

Under these conditions and in the dark, the intracellular injection of either cGMP or EGTA produced an increase in the inward current. The drug-induced increase in current was clearly light-sensitive since it was totally suppressed by bright illumination. With lengthier injections of these substances, increasingly brighter flashes were necessary to totally suppress the current.

The reversal potential of the cGMP- or EGTA-induced current was the same as the normal light-sensitive current. Hence, the ionic basis of the drug-induced currents and of the light-sensitive current appeared to be identical. The finding that either an increase in cGMP or a decrease in calcium can enhance the light-sensitive current supports the notion that both substances are among the possible internal messengers in rod outer segments. (Supported by a John A. and George L. Hartford Foundation Fellowship and National Institutes of Health Research Grant EY00606).

258.3 THE OUTER SEGMENT MEMBRANE CURRENT OF SINGLE GREEN ROD PHOTORECEPTORS FROM TOAD RETINA. G. Matthews. Department of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Amphibian retina contains two types of rod photoreceptor: red rods and green rods, named for their apparent color when a flat mount of fresh retina is viewed in white light (Denton & Wyllie, J. Physiol., 1955, 127: 81-89). Little is known of the physiology of green rods, primarily because they make up only about 10% of the total rod population and are thus encountered only rarely in intracellular recording from intact retina. In order to compare the properties of green and red rods, recordings were made of the outer segment membrane current of single green rods from the retina of the toad, Bufo marinus. This animal was chosen because its red rods have been particularly well-studied and because its red and green rods may be easily distinguished by visual inspection. All manipulations were conducted under visual control on the stage of a compound, inverted microscope equipped with an infrared-sensitive TV camera. Membrane current was recorded using the suction-electrode technique of Baylor, Lamb & Yau (J. Physiol., 1979, 288: 589-611).

The dim-flash response of green rods was similar in form to that of red rods, i.e., in most cases the response could be fitted by an expression for the impulse response of a series of four low-pass filters. Double logarithmic plots of response amplitude divided by light intensity against time after a brief flash had an initial slope of about three, as would be expected if the initial rise were limited by propagation through four sequential delay stages. Thus, four stages of delay seem to be characteristic of the phototransduction process in rat rods (Penn & Hagins, Biophys. J., 1972, 12: 1073-1094), in toad red rods (Baylor et al., 1979) and in toad green rods.

The relation between peak response amplitude and flash intensity could be fitted by a rectangular hyperbola with average half-saturating intensity of 2.75 photons μm^{-2} (N=6). After correction for the use of unpolarized light in the present work, this is similar to the value of 1.46 photons μm^{-2} reported for toad red rods by Baylor et al. (1979).

Spectral sensitivity curves for green rods were determined for wavelengths between 400 and 620 nm, corresponding to a sensitivity range of about 6 orders of magnitude. Fitting the new Dartnall nomogram for vitamin-A_1-based pigments to the data yielded a maximally absorbed wavelength of 433 nm, in close agreement with previous estimates from microspectrophotometry (Harosi, J. gen. Physiol., 1975, 66: 357-383).

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258.4 LIMULUS PHOTORECEPTORS RESPOND WITH A REDUCED LATENCY WHEN PHOTONS ARE EFFECTIVELY ABSORBED LESS THAN A MICRON APART. Richard Payne* and Alan Fein* (SPON: E.F. MacNichol, Jr.). Marine Biol. Lab. Woods Hole MA 02543.

Observations of the linear nature of the response of invertebrate photoreceptors at early times following weak or moderately intense flashes have often been taken to imply that the initial stages of transduction constitute a linear biochemical system of cascaded enzymes. However, the stimuli used may not have been sufficiently intense to evoke non-linear interactions between intermediates released from adjacent sites of photoisomerization (Rh*), if those intermediates diffuse less than a few microns during the latent period of the response. To achieve higher densities of Rh*, light may be focused onto a small patch of rhabdom.

10-20 μm diam. spots of light were focused onto the most sensitive regions of Limulus ventral photoreceptors bathed in ASW at 20° C. The photocurrent generated by 10 ms flashes was recorded with a conventional voltage clamp holding the cells at their resting potentials. The averaged responses of dark-adapted cells showed substantial supralinearity at the earliest times of response. A measure of this supralinearity is the time, Δt, taken for the response to exceed 0.003nA per Rh*. Δt should be a constant for a linear process. In the present experiments, Δt fell from typically 100 to 50ms as the number of Rh* produced by the flashes was increased from 100 to 10,000. Below 100 Rh* the response was linear, so that Δt was unchanged. Adaptation with a diffuse background, that reduced the sensitivity of the cell to the spot by 1 log unit, had no effect on the intensity range of flashes over which Δt fell. Responses to flashes of diffuse illumination showed similar reductions in Δt for flashes producing $>$1000 Rh*. The effect of flash intensity on Δt therefore appears to be a function of the density of Rh*, rather than the absolute numbers of Rh* or the cell's sensitivity.

The results suggest the existence of an early non-linear step in invertebrate transduction mediated by diffusion over a distance of <2 μm from the site of a photoisomerization. The supralinearity of the early response indicates that an autocatalytic or cooperative reaction may be involved. The possibility that the response to single photons is generated by localized non-linear elements in the invertebrate photoreceptor merits further experimental and theoretical consideration.

258.5 PENETRATION OF A FLUORESCENT DYE INTO LIMULUS VENTRAL PHOTORECEPTOR RHABDOM. P. H. Hartline. Eye Research Institute of Retina Foundation, Boston, MA 02114

The fluorescent dye didansyl cystine (DDC), widely used to assess integrity of vertebrate rod outer segment membranes, is also useful for exploring cellular distribution and accessibility of invertebrate rhabdom. The dye binds to membrane but does not cross it and enter cytoplasm. To test DDC's binding to invertebrate membrane, a thin ribbon of ventral olfactory nerve fibers was exposed to 2.5 - 25 μM DDC. Fluorescence rapidly attained equilibrium (within a few minutes), and was proportional to DDC concentration in the bathing saline. Ventral photoreceptors similarly exposed to DDC acquired fluorescence very slowly. Usually, fluorescence increased linearly over at least an hour, showing saturation in only a few cells. Eventually, some regions of photoreceptors become brightly fluorescent, presumably because they are packed with rhabdomal microvillar membrane.

The dye must penetrate into the rhabdom by diffusion within the extracellular spaces of the rhabdom or in clefts between photoreceptor and glial membrane. The rate at which DDC accumulates in rhabdomal membrane is determined by its diffusion constant, by the cross sectional area of the spaces within which diffusion is confined, and by partition of the dye between extracellular fluid and the adjacent membrane. If rhabdomal membrane is freely accessible through large extracellular clefts, equilibrium fluorescence should be attained rapidly, as in nerve fibers. Since very slow DDC uptake was observed in the photoreceptors, it appears that extracellular space in the rhabdom, or in other access channels, must be severely restricted.

Odette and Hartline (Biophys. J. 137: 274a, 1982) have proposed that the rhabdom acts as an electrical filter (a cable). They calculated that the rhabdomal cable substantially slows electrical events that originate in microvillar membrane but are measured in the cell body, assuming that extracellular space is sparse in the rhabdom, and that there are not large extracellular access channels. These assumptions are supported by the slow accumulation of DDC by ventral photoreceptors.

After a suitable exposure to DDC, ventral photoreceptors have distinctly fluorescent subregions; some of these are at or near the cell's surface, and others appear to lie within the boundaries containing the cell. In three dimensions, the boundaries of fluorescent regions follow features that are visible in transillumination. That cells exposed to DDC remain healthy is suggested by their normal physiological responses to light following long exposures (several hours) to 100 μM DDC. Thus, DDC may provide a convenient way to visualize rhabdomal regions of living invertebrate photoreceptors.

258.6 CALCIUM AND LIGHT-EVOKED CONTRACTION OF THE PHOTOSENSITIVE IRIS OF THE FROG. G. J. Kargacin* and P. B. Detwiler* (SPON: G. Balkema). Dept. of Physiol. Biophys., Univ. of Wash. Sch. of Med., Seattle, WA 98195.

The smooth muscle cells of the iris sphincter pupillae of some vertebrates contain rhodopsin and contract as a result of the direct action of light upon them. The mechanism of this light-evoked contraction is not known. Since smooth muscle contraction is generally thought to be triggered by an increase in intracellular calcium and since light-evoked release of calcium has been demonstrated in rod photoreceptors, we have chosen to investigate the role of calcium in the light-evoked contraction of the frog (*Rana pipiens*) iris.

The calcium ionophore A23187 (10^{-5}M) and carbacol (10^{-5}M) both produce a calcium-dependent contraction of the sphincter when they are present in solutions bathing the iris. Contractions produced by high potassium solutions (50mM KCl) have a similar calcium dependence. These results indicate that the contractile proteins of the iris sphincter can be activated by an increase in intracellular calcium.

Smooth muscle cells near the pupillary margin of the iris were impaled with intracellular microelectrodes and simultaneous measurements of the membrane potential of these cells and of the contractile tension of the sphicter were made. Pupillary cells had resting potentials of about -60mV. No electrical potential changes were seen prior to the onset of light-evoked tension. The potential changes seen during contraction appeared to be artifacts due to movement. The apparent lack of involvement of external ions in the iris light-response suggested by these experiments was further supported by results that showed that the iris continued to contract in response to light in sodium-free solutions, calcium-free solutions, and high-potassium depolarizing solutions (110mM KCl).

From this study, we conclude that light directly activates contraction of iris smooth muscle cells by a mechanism that does not involve a measurable permeability change of their surface membrane, and that is not immediately dependent upon external calcium. The contractile proteins of the muscle can be activated by calcium, however, suggesting that an internal calcium store might be involved in the light response.

258.7 ON-LINE COMPUTERIZED ANALYSIS OF NEURONAL THERMOSENSITIVITY, B. Krespan*, N.L. Geller*+, H.M. Geller, Dept. of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854, +Biostatistics Laboratory, Memorial-Sloan Kettering Cancer Center, New York, NY 10021. Neurons in several areas of the nervous system respond to an alteration in local temperature by a change in firing rate (FR). Such neurons have been classified as thermosensitive based on either an estimate of Q10 (the ratio of change in FR over a 10°C interval) or the slope of the thermal response curve. We have developed and implemented a computer program for the on-line collection, display and statistical analysis of thermal response data. The program is written in FORTRAN except for routines which interface to the experimental apparatus. Temperature is measured by sampling the amplified output of a YSI telethermometer and digitizing it using the internal A/D converter; a calibration routine for each YSI probe is included. During data collection, interspike intervals (ISI) are measured by an interrupt driven routine which triggers A/D conversion to obtain the corresponding temperature. A point plot of instantaneous FR (computed as 1/ISI) versus temperature is updated for each spike. The data is stored on disk allowing further off-line analysis. The program was tested by characterizing the thermal response of eight neurons in cultures of medial basal hypothalamus. The distribution of FR was very skewed and hence not normally distributed. A logarithmic transformation of the firing rate was deemed appropriate to reduce skew; least squares regression was then applied. For each cell we first tested the hypothesis of no correlation between temperature and the transformed FR. If this hypothesis was not rejected, we concluded that the cell was not temperature sensitive. If it was rejected, we propose an estimator of Q10. This is a maximum likelihood estimator if the transformed data satisfy the assumptions of ordinary least squares regression. From confidence belts for estimated FR, conservative lower and upper 90% confidence limits for Q10 are derived. Neurons with lower confidence limits for Q10s greater than 2 are considered warm sensitive; those with upper confidence limits less than $\frac{1}{2}$ are considered cold sensitive; all others are considered temperature insensitive. Our initial results showed that in 4 cells there was no correlation between FR and temperature; these cells were thus classified as temperature insensitive. In 4 cells there was a correlation between FR and temperature at a 90% significance level. The Q10 of 3 of these cells was between 0.5 and 2.0; these cells also were not temperature sensitive. In the final neuron of this series the Q10 was 3.2, with lower confidence limit 2.0 and upper confidence limit 5.4, thus meeting the criterion for warm-sensitivity. Studies of other parameters are planned in both MBH and POAH cultures. (Supported by NSF grant BNS 81-16434.)

258.8 EFFECTS OF CALCIUM BLOCKERS (Mn^{2+} AND VERAPAMIL) ON THE RESPONSE OF CAT CAROTID CHEMORECEPTORS. A. Gual* (SPON: C. Eyzaguirre). Dept. Physiol., Univ. Utah School Med., Salt Lake City, Utah 84108.

The excised carotid body and its nerve were superfused in vitro with modified Tyrode's solution equilibrated with 50% O_2 in N_2, pH 7.43 at 35°C. Adding Mn^{2+} (0.3 mM to 3.0 mM) progressively decreased and then blocked the discharges. When Mn^{2+} substituted Ca^{2+}, discharges were blocked but reappeared on returning to control solution. Adding Verapamil (V) 1 μg/ml increased the discharge frequency (over 30%) which returned to baseline in control solution. When superfusion with V 1 μg/ml was maintained for more than 30 min, the discharges decreased moderately. During V superfusion, the carotid body responded normally to acetylcholine (ACh) and dopamine (DA) with a respective increase and decrease of the discharge. Adding V 10 μg/ml a moderate increase of discharges was followed by depression. Also, the responses to ACh and DA were unaffected.

These experiments suggest that small doses of V (calcium entry blocker) may have an excitatory effect on the glomus cells. This effect could be produced by inhibition of calcium mitochondria uptake. As consequence, the cytoplasmatic free calcium would increase mediating the excitatory effects.

The inhibitory effect of larger doses of V may be attributed to its anesthetic effect (5.1 x Lidocaine) on the fibers, probably through its known interference with the Na^+ current. Mn^{2+} probably blocked Ca^{2+} channels in both directions and/or exocytosis of excitatory or inhibitory modulators from glomus cells.

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258.9 A CHEMICAL MODEL FOR THE BINDING OF d-TUBOCURARINE TO VOLTAGE DEPENDENT MECHANORECEPTOR CHANNELS IN THE PROTOZOAN, STENTOR.

D.C. Wood. Psychobiology Program, University of Pittsburgh, Pittsburgh, PA 15260.

The ciliate protozoan, Stentor coeruleus, generates a graded depolarizing mechanoreceptor potential whose amplitude (< 25 mV) is a function of mechanical stimulus intensity. This potential is produced by a transient (< 40 msec) increase in membrane conductance and a resultant inward Ca^{++} flux. Depolarizing the cell above its normal -50 to -55 mV resting potential increases the receptor current though decreasing its driving force; hyperpolarizing the cell decreases the receptor current though increasing the driving force. The mechanoreceptor channel is therefore voltage dependent. This voltage dependence can be most simply described by assuming that the channel has two possible forms: an R form which is responsive to mechanical stimuli and a U form which is unresponsive to mechanical stimuli. The ratio of channels in these two forms is a simple exponential function of the transmembrane potential (21 mV/e-fold change).

Mechanoreceptor currents elicited from cells at their normal resting potential are effectively blocked by d-tubocurarine (dTC) in a dose dependent fashion (ED_{50} = 7.5 μM). dTC at these concentrations does not significantly effect the resting potential, action potential peak, membrane resistance or mechanoreceptor current reversal potential. The effects of dTC on the mechanoreceptor current are well correlated with the binding of ^{14}C dTC to the cell surface, suggesting that dTC binds rather specifically to the mechanoreceptor channels.

Three lines of evidence demonstrate that dTC binding interacts with the molecular mechanism producing voltage dependence. Firstly, large depolarizing voltage steps applied to cells bathed in a dTC containing solution temporarily reduce the blockade of the mechanoreceptor current produced by the drug. Secondly, the blockade of mechanoreceptor current produced by dTC in control medium is reduced if the cells are depolarized by 2-8 mM KCl solutions. Thirdly, the binding of labelled dTC is inhibited by placing cells in these same depolarizing KCl solutions.

A series of quantitative models have been developed and fit to the curves describing voltage dependence, dTC binding and the interaction between these two processes. Adequate fits are obtained only for models which assume that: 1) dTC binds with higher affinity to the U form than to the R form. 2) the R form can conduct current even when dTC is bound to it. 3) a given change in V_m produces a larger shift between U-dTC and R-dTC than between U and R.

On the basis of this analysis it can be suggested that the voltage dependence of the mechanoreceptor channel is the result of movement of a positively charged element.

259.1 CHANGES OF INTRACELLULAR SODIUM AND POTASSIUM IN FROG MOTONEURONS INDUCED BY REPETITIVE SYNAPTIC STIMULATION.

G. ten Bruggencate, P. Grafe*, M. M. Reddy* and J. Rimpel*
Department of Physiology, University of Munich, Germany

Double barrelled ion selective microelectrodes and conventional microelectrodes were used to measure the membrane potential, the free intracellular Na^+- and K^+-concentrations (Na^+_i; K^+_i), the extracellular K^+-concentration K^+_e, and the input resistance of lumbar motoneurons in the isolated frog spinal cord. Observations were made under resting conditions, and both during and after repetitive (10/s) stimulation of a dorsal root. The following results were obtained:

1. K^+_i was between 80 and 105 mmol/l in neurons with resting membrane potentials of -70 to -80 mV and action potentials of 90 - 105 mV amplitude.
2. Na^+_i was between 4 and 16 mmol/l in motoneurons having resting membrane potentials of -75 to -65 mV.
3. Upon repetetive (10/s) stimulation for 10s, K^+_i fell by upto 5 mmol/l and Na^+_i rose by upto 5 mmol/l. Following stimulation, it took several minutes until K^+_i and Na^+_i returned to their baseline values.
4. Following stimulation, the membrane potential showed a post-tetanic hyperpolarization (PTH). At this time, K^+_i and Na^+_i had their lowest and highest values, respectively.
5. Since K^+_e was still elevated at the time of the PTH, the membrane potential did not passively follow the transmembrane K^+-distribution. During the PTH, which could be blocked by ouabain, no change in membrane resistance could be detected.
6. In the presence of 15 mmol/l LiCl, the PTH was abolished and the recovery of K^+_i after stimulation prolonged. Also, LiCl reduced K^+_i, suggesting that an important part of Li action may be mediated via a change in the K^+ gradient.

It is concluded that transient changes in K_i and Na_i result from repetetive synaptic activity, and that such changes can be detected by intracellular ion selective microelectrodes. The PTH is probably due to an electrogenic action of the sodium pump, which in turn is activated by the transient rise in Na_i. In addition to its action on the transmembrane K^+ gradient, lithium blocks the PTH.

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259.2 MEASUREMENTS OF INTRACELLULAR $[Ca^{2+}]$ IN CULTURED MAMMALIAN NEURONES. M. E. Morris and J.F. MacDonald (SPON: I. C. Bruce). Playfair Neuroscience Unit and Departments of Anaesthesia and Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

Liquid ion-exchanger microelectrodes were used to record the level of [free Ca^{2+}] in neurones from mouse spinal cord and dorsal root ganglion, which had been grown in dissociated tissue culture. Electrodes were pulled from θ-capillary tubing with tip diameters ≤0.5 µm. One channel, silanized with the technique of Coles and Tsacopoulos (*J. Physiol. 270: 13-14P, 1977)*, contained a column of liquid Ca^{2+} sensor *(Oehme et al., Chimia 30: 204-206, 1976; Lanter et al., Anal. Chim. Acta 135: 51-60, 1982)* at the tip and 0.1 M KCl, buffered to 10^{-7} M $[Ca^{2+}]$. The second channel was filled with 3 M KCl, had resistance = 12-40 MΩ, and was used to measure membrane potential as well as for differential recording of Ca^{2+} potential (V_{Ca}). Rejection capacity of the electrode and amplifier to 100 mV input between bath and ground was ≥98%. When electrodes were calibrated in buffered solutions of 10^{-2} to 10^{-7}M $[Ca^{2+}]$ in 0.1 M KCl, slopes were 25-42 mV and the limit of detection was between 10^{-6} and 10^{-7} M $[Ca^{2+}]$. During observations neurones were bathed in HANKS balanced salt solutions at pH 7.4 and osmolarity 330 mosmol, which contained either 1.3, 3, or 6 mM Ca^{2+}, and 0.9 mM Mg^{2+}, 142 mM Na^+, 5.8 mM K^+, 145.9-154.3 mM Cl^-, 4.2 mM HCO_3^-, 0.7 mM HPO_4^-, 25 mM HEPES, and 600 mg% glucose. Drugs were applied by pressure ejection or microperfusion from pipettes with tip size of 2-10 µm at a distance of 10-50 µm from cells. Under direct visualization recordings were made in 40 neurones of ≈ 15-40 µm in diameter. On electrode entry V_{Ca} usually fell with half-times of 1-6 s and remained relatively stable during periods of 1-120 min (mean duration = 7 min), with recovery half-times of 3-12 s. In 29 neurones the mean level of $[Ca^{2+}]_i$ was 1.89×10^{-5} (S.D.± 2.63×10^{-5}) M; in 11 other cells values were between 1.2 and 8×10^{-4} M. The membrane potential measured at the same time was -24.8±11.7 mV (mean±S.D., n = 40). In most recordings at the time when V_{Ca} was at or near stabilized levels there was progressive depolarization with shunting of E_m, attributable to leakage around the electrode tip. Spikes usually inactivated rapidly, although in some cases hyperpolarizing current produced recovery. In 7 of 12 spinal cord neurones synaptic activity was present. During applications of L-aspartate (0.5 - 1 mM) no changes in $[Ca^{2+}]_i$ were observed, even when ↑E_m and ↑G_m were recorded. Sustained, rhythmic fluctuations in $[Ca^{2+}]_i$ (rise time 15 s, period = 300 s, $\Delta[Ca^{2+}] = 2.8 \times 10^{-4}$ M) were, however, seen during perfusion with 125 mM TEA. The success of these preliminary measurements of cytoplasmic $[Ca^{2+}]$ in small neurones strongly encourages further technical improvements and experimentation. *(Supported by The Medical Research Council of Canada).*

259.3 EFFECTS OF INTRACELLULAR PRESSURE INJECTIONS OF CALCIUM IONS IN MORPHOLOGICALLY IDENTIFIED NEURONS OF CAT MOTOR CORTEX.

R.A. Wallis*, C.D. Woody and E. Gruen* (SPON: B. Swartz).
Departments of Anatomy and Psychiatry, UCLA Medical Center, Los Angeles, CA 90024.

Injections of calcium ions and Horseradish Peroxidase (HRP) were made intracellularly in neurons of the motor cortex of awake, unparalyzed cats. Pressure injection was used to avoid voltage dependent calcium flux that could arise from iontophoretic application. The methods of intracellular recording and pressure injection have been previously described (Woody and Black-Cleworth, J. Neurophysiol., 1973; Sakai et al, Neuropharmacol., 1979). Twelve cells with resting potentials averaging 49 mV were injected with 4% HRP in 10^{-3} M $CaCl_2$. Twenty-nine cells injected with HRP without additional calcium had resting potentials averaging 47 mV. Injections with calcium produced an increase in conductance measured by the differential spike height method that was usually sustained over the course of 3 minutes. Injections of HRP alone produced no change or significantly smaller conductance increases. HRP solutions were found to contain as much as 10^{-4}M free calcium ions. Cells responding to calcium with a conductance increase were identified as pyramidal cells in layers II, III and V. The effect of increasing intracellular calcium resembled that found in spinal motoneurons (Krnjevic and Lisiewicz, J. Physiol., 1972) and in some types of invertebrate neurons (Heyer and Lux, J. Physiol., 1976; Meech and Strumwasser, Fed., Proc., 1970). (Supported by AGO 1754, HD 05958, and AFOSR 81-0179).

259.4 INTRACELLULAR RECORDING IN THE MOLECULAR LAYER OF SLICES OF THE DENTATE GYRUS MAINTAINED IN VITRO. J.M. Godfraind*
(SPON: M. Meulders). Lab. Neurophysiologie, Faculté de Médecine, UCL 5449, B-1200 Brussels, Belgium.

Recently, in dentate gyrus slices, intracellular recording performed in the granule cell layer has shown that, in addition to the usually expected spike potentials, "small amplitude spikes" (SA spikes) can be evoked in some conditions (Assaf et al. in Electrophysiology of the isolated mammalian CNS preparations, pp 153, Eds. Kerkut and Wheal, Pergamon, 1981). These SA spikes could represent the intracellular counterpart of the dendritic electrical activities recognized by field potential analysis (Jefferys, J. Physiol, 289: 375, 1979).

In this study, fine micropipettes have been directed to the granule cell layer and, for comparison, to the molecular layer of the dentate gyrus — which contains the dendritic arborization of the granule cells — to see if so-called "dendritic activities" could be recorded intracellularly at this level.

Transverse slices of the hippocampus containing the dentate gyrus were taken from the brain of rats, maintained in vitro and perfused according to techniques previously used (Kelly et al., Brain Res., 168:388, 1979). After impalement with micropipettes filled either with 3 MKcl or 1 MK acetate, neuronal elements were simply stimulated by passing depolarizing and hyperpolarizing current pulses through the recording electrode.

In the granule cell layer, observations were similar to those reported (Assaf et al.,1981): on 6 neurones impaled with a mean resting potential of 42 mV (SD= ± 11.2) and a mean spike amplitude of 40.3 mV (SD= ± 16.5),only two exhibited SA spikes whose amplitude represented at most 20 to 29% of that of the action potentials.

In the molecular layer, on 6 units encountered with a mean resting potential of 49.3 mV (SD= ± 12.8) and a mean spike amplitude of 36.6 mV (SD= ± 7), all presented SA spikes whose mean amplitude was 20.8 mV (SD= ± 6.52) and represented 57% of that of the spikes. Three types of SA spikes were noted. Most often, they were associated with a spike in a "pair", the SA spike being the second; they appeared inside a burst of action potentials evoked by a strong depolarizing pulse; or they occured independently. Other neuronal elements were also extracellularly and intracellularly recorded without apparent SA spikes. In conclusion, SA spikes can be recorded in the molecular layer of the dentate gyrus. Whether or not these potentials represent intradendritic recording of granule cells or other neurones, can only be answered by a combined electrophysiological-morphological approach (Wong et al., Proc.nat.Acad.Sci., 76:986, 1979).

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259.5 CHOLINERGIC ENHANCEMENT OF PENICILLIN-INDUCED EPILEPTIFORM DISCHARGES IN PYRAMIDAL NEURONS OF THE GUINEA PIG HIPPOCAMPUS. A.R. Kriegstein*, T. Suppes, and D.A. Prince. Dept. of Neurology, Stanford Medical Center, Stanford, CA 94305.

Acetylcholine (ACh) increases excitability of the hippocampus by a variety of mechanisms, including increases in input resistance and depolarization of hippocampal pyramidal cells (HPCs) and depression of IPSP's and slow afterhyperpolarizations (AHPs). Penicillin disinhibits HPCs and leads to the development of epileptiform bursting. Experiments were done in guinea pig hippocampal slices to determine whether cholinergic excitation would enhance penicillin epileptogenisis.

Field potentials were evoked in the CA_1 region by stimulation of stratum radiatum. ACh (1-20 mM) was pressure ejected adjacent to the recording electrode in stratum pyramidale. Focal application of ACh to slices bathed in normal Ringer's produced an increase in complexity, amplitude, and duration of the evoked field potential which returned to normal within 5-20 sec (n=5). Penicillin perfusion (3.5 mM) led to the development of spontaneous and evoked multiphasic epileptiform field potentials lasting 20-100 msec. The complex field potentials produced by ACh alone were enhanced and prolonged dramatically in the presence of penicillin. The enhancement consisted of a 2 to 10 fold increase in the number of peaks in the multiphasic field potential and an increase in response duration to over 100 msec. Maximal changes occurred in 10-20 sec and responses usually returned to baseline values within 120 sec (n=15), but in some cases remained enhanced up to 4 minutes (n=5). Simultaneous intra- and extracellular recordings demonstrated that the enhanced field potentials were associated with prolonged membrane depolarizations and repetitive firing.

Exposure to both agents leads to a greater enhancement of excitation in HPCs than either drug produces alone, presumably because of summation of disinhibitory effects and the additional direct effect which ACh has on intrinsic membrane properties. Cholinergic input to the hippocampus may act to amplify ongoing epileptogenic activity.

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259.6 INTRACELLULAR RECORDINGS OF PHASIC BURSTING FROM NEUROENDOCRINE CELLS IN SLICES OF RAT HYPOTHALAMUS. R.D. Andrew and F.E. Dudek. Dept. Physiol.,Tulane Univ. Sch. Med., New Orleans, LA 70112.

Extracellular recordings from magnocellular neuroendocrine cells (MNC's) in the rat suggest that phasic burst firing is associated with increased release of vasopressin from the posterior pituitary. Intracellular recordings, which permit direct study of intrinsic and synaptic mechanisms underlying firing patterns associated with hormone release, have proven difficult previously in vivo. Thus, we have used the rat hypothalamic slice to impale MNC's in supraoptic (n=11) and paraventricular (n=4) nuclei. Stable recordings (10-150 min) revealed cells with spike amplitudes of 70-95 mV, input resistances of 55-200 MΩ and repetitive spiking during depolarizing current injection. Of 15 cells, 5 fired phasic bursts at normal or slightly hyperpolarized membrane potential (E_m). Increased hyperpolarizing current injection (0.10-0.15 nA) could eliminate phasic bursting and the accompanying slow oscillations of E_m; furthermore, patterned synaptic potentials were not observed (n=5), although synaptic bombardment induced one of many bursts in one phasic burster. Increased depolarization (or reduced hyperpolarization) of E_m lead to longer bursts and higher intra-burst spike frequency to a point where spiking became continuous. Also, brief pulses of injected current could dramatically alter firing patterns over many minutes. These observations suggest an endogenous pacemaker mechanism within some MNC's.

Phasic MNC's invariably displayed progressive spike broadening during each burst, suggesting increased Ca^{2+} influx during consecutive action potentials of a single burst. A current-evoked spike train induced a subsequent hyperpolarizing afterpotential (HAP), which a number of observations suggest is due to a Ca^{2+}-dependent K^+ conductance (R.D. Andrew and F.E. Dudek, 1982, Fed. Proc. 4:1354). The HAP was occasionally followed by a depolarizing afterpotential (DAP), which often lasted several seconds and had superimposed spikes. The HAP-DAP sequence was not blocked by tetrodotoxin (which eliminated Na^+ but not Ca^{2+} spikes). In one phasic cell, the DAP following each spontaneous spike obviously contributed to a slow depolarization underlying each burst. However, in another cell a slow depolarization could occur in the absence of spikes. Thus, the slow depolarization of E_m during a burst can be promoted by, but is not necessarily dependent on, cell firing. We conclude that in some MNC's a spike burst can activate an oscillatory mechanism involving intrinsic membrane conductances, which probably contribute to phasic bursting.

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259.7 A STATE-DEPENDENT, SLOW THALAMIC RHYTHM. M.Deschênes*,G.Oakson*, J.P.Roy* and M.Steriade Lab. of Neurophysiology, Dept of Physiology,Fac. of Medicine, Université Laval, Québec, CANADA, G1K 7P4.

Intracellular recordings of thalamic neurons (VL-VPL-CL nuclei) were performed in cats under barbiturate anesthesia. In all thalamic neurons two coexistent rhythms were observed. Brief episodes (1-2 sec) of membrane potential oscillations at frequencies of 8-12 Hz appeared with a periodicity of about 10 seconds. In relay neurons, each episode was characterized by a sequence of hyperpolarizations and burst discharges. These rhythmic episodes of hyperpolarization recurring about every 10 seconds could be reversed in sign by hyperpolarizing currents or by Cl injection hence, suggesting that they were mainly composed of rhythmic IPSPs. This result also indicated that the slow 0.1 Hz rhythm was imposed on the relay neurons by other neuronal pools. Following a complete isolation of the thalamus by cortical and high brainstem lesions the slow 0.1 Hz rhythm was still present and it was concluded that this rhythm was generated within the thalamus by local interneurons. Thalamic interneurons (identified by electrophysiological criteria) behaved exactly as one might expect from pacemaker cells. Brief episodes (1-2 sec) of repetitive depolarizations (8-12 Hz) and burst discharges recurred every 10 seconds in these cells. In the interval, the membrane potential of these interneurons slowly hyperpolarized contrasting with the rhythmic phasic hyperpolarizations observed in relay neurons. Statistical analyses of the spontaneous firing of thalamic neurons in chronic behaving preparations also displayed fluctuations of cellular excitability approximately every 10 seconds. These rhythmic fluctuations were very proeminent in slow wave sleep, they tend to disorganize in the pre-arousal period and, during periods of quiet wakefulness, they were still present but largely attenuated. In acute barbiturated preparations high frequency stimulation of the mesencephalic reticular formation disrupted the slow rhythm for periods outlasting the duration of stimulation. From the above results, it is concluded that at rest, in a state of functional deafferentation (sleep or barbiturate anesthesia) the membrane potential of thalamic neurons oscillated at a frequency of ≈0.1 Hz and that this slow rhythm is generated through the interaction of intrinsic membrane properties and interneuronal connectivity.

259.8 THE ORIGINS OF PRIMARY AND SECONDARY SYNCHRONIZED BURSTS IN DISINHIBITED HIPPOCAMPAL SLICES, Richard Miles* and Robert K.S. Wong (SPON: J.P. Baker). Department of Physiology and Biophysics, Univ. of Texas Medical Branch, Galveston, TX 77550.

Hippocampal pyramidal cells fire in rhythmic synchronized bursts when GABA-ergic inhibition is blocked. To investigate this phenomenon intracellular and field potential recordings were made from Vibratome-cut guinea-pig hippocampal slices exposed to 10^{-4} M picrotoxin.

Synchronized field potential activity occurred spontaneously at frequencies from 0.08-0.2 Hz in all pyramidal cell areas or could be evoked by stimulation of the fimbria or mossy fibres. Intracellularly the activity consisted of a primary burst, several spikes followed by a plateau depolarization of up to 45 mV lasting 80-160 msec. This was followed by from 1-6 secondary bursts, one or two spikes on the rising phase of a round depolarization of up to 35 mV and of duration 30-60 msec, at latencies of 100-300 msec from the start of the primary burst.

Spontaneous primary bursts usually occurred first in CA2. The intracellular stimulation of a single cell here can reset the rhythm of the field potential discharge supporting the hypothesis that CA2 normally initiates synchronized activity.

Both primary and secondary bursts depend on intact chemical synaptic transmission. Electrotonic transmission, with coupling ratio 0.1-0.2, between CA2 neurones has been observed in 5 out of 53 paired intracellular recordings. However this is not sufficient to cause synchronized activity since both spontaneous and evoked bursts were blocked in a low Ca (0.5 mM)-high Mg (8 mM) solution.

Cuts were made orthogonal to the pyramidal cell body layer and extending from the alveus to the hippocampal fissure in order to investigate the origin of the synchronized discharges. When CA2 was separated from CA3 spontaneous bursts occurred independently with a different rhythm in each area. In CA2 primary bursts occurred alone at a frequency of 0.15-0.3 Hz. The activity in CA3 consisted of primary bursts followed by secondary bursts at a lower frequency of 0.04 to 0.08 Hz.

It therefore seems that there is a functional differentiation of hippocampal pyramidal cells into areas including a pacemaker region which normally initiates primary synchronized bursts and a separate region where secondary synchronized bursts are generated. This may be dependent on differences in the properties of single pyramidal cells or on the ways in which they are connected in these two areas.

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259.9 ETHANOL ALTERED EXCITABILITY IN HIPPOCAMPAL CA3 NEURONS RECORDED IN VITRO. K. L. Zbicz* and F. F. Weight (SPON: M. J. Eckardt). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The effects of ethanol on the excitability of mammalian central neurons was studied in vitro using electrophysiological techniques. Thin slices of guinea pig hippocampus (400-450 u) were submerged in a chamber and superfused by an oxygenated, rapidly flowing artificial CSF solution at 33°C. Extracellular recording was used to monitor the spontaneous firing of neurons in the stratum pyrimidale layer of region CA3. Superfusion of the slices with solutions containing 30-200 mM ethanol consistently produced a dose dependent increase in the spontaneous firing rate of the neurons. However, ethanol in concentrations 300 mM or greater produced a depression of spontaneous firing. A similar effect of ethanol has been observed in rat hippocampus in vivo (Grupp, L.A. and Perlanski, E., Neuropharmacol. 18: 63, 1979).

The mechanisms involved in the excitatory effects of ethanol were studied using intracellular techniques. With intracellular recording, application of 30-200 mM ethanol also produced increased spontaneous firing. Depolarization of the membrane by either step or ramp currents induced the generation of action potentials. The number of spikes elicited by such depolarizations was increased in the presence of 30-200 mM ethanol. In addition, the minimum current needed to initiate action potentials (viz. threshold) was was decreased. The current-voltage relationships of the neurons were studied using both current-clamp and voltage-clamp methods (Wilson, W.A. and Goldner, M., J. Neurobiol. 6: 411, 1975). No changes in the I-V curves were detected in the presence of 30-200 mM ethanol at potentials negative to threshold. After the washout of ethanol, there was a depression of both spontaneous activity and the number of spikes elicited by depolarizing current. The minimum amount of current needed to activate action potentials was elevated during this period of depression. Following the period of depression, spontaneous firing and responsiveness returned to near pre-ethanol levels.

The results indicate that the administration of ethanol in concentrations greater than 30 mM alters the excitability of hippocampal neurons. Our data suggest that the increased excitability that was observed results from a change in spike threshold rather than a change in membrane conductance.

259.10 HIPPOCAMPAL SLICE EXCITABILITY: COMPARATIVE EFFECTS OF VARYING Ca^{2+}, Mg^{2+} and K^{+} CONCENTRATIONS AND DURATION OF INCUBATION. T.A. Pitler*, G. Morgan*, P.W. Landfield (SPON: J. McCormick). Dept. of Physiol. and Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

It is well established that variations in extracellular K^{+} or the Ca^{2+}-Mg^{2+} ratio affect membrane excitability and synaptic potentials in brain slices. Moreover, excitability in slices can gradually alter over time. However, these variables have rarely been systematically compared within the same studies. The present report describes parametric studies aimed at clarifying the interrelations among these factors and at gaining greater experimental control over slice preparations.

In each experiment, slices from a single rat were incubated simultaneously in 4 wells of a static bath slice chamber. Each well contained a different composition of artificial cerebrospinal fluid (ACF), allowing slices from the same rat to be compared across the various ACF media. Five media compositions were studied: High Mg^{2+}-to-Ca^{2+} ratio, with either low K^{+} or high K^{+}; equal Mg^{2+}-to-Ca^{2+} ratios, with low or high K^{+}; and a high Ca^{2+}-to-Mg^{2+} ratio, with high K^{+}. Approximately 90 slices from 15 rats were examined in this study. Input-output curves for population spikes and population EPSPs were recorded simultaneously with two extracellular pipettes (in somal and dendritic layers, respectively) in response to stimulation of the Schaffer-commissural radiatum afferents.

Spike amplitudes as a function of stimulation were considerably larger in high K^{+} vs low K^{+} media, when other ions were constant. This effect was primarily due to an increase in the spike-to-EPSP ratio, and therefore presumably to increase membrane excitability. Increased spike amplitudes as a function of stimulation were also seen in the higher Ca^{2+}-to-Mg^{2+} ratio media. However, this effect was primarily due to an increase in the input-output curve of the EPSP. The largest absolute values of population spikes and EPSPs were obtained in the high Ca^{2+}, high K^{+} media, whereas, the lowest values were found in high Mg^{2+}, low K^{+} media. These results indicate that the effects of Ca^{2+}-Mg^{2+} balance on synaptic potentials outweigh their effects on somal excitability, within these moderate ranges, and that the effects of K^{+} and of Ca^{2+}-Mg^{2+} balance are roughly additive.

Slices that were incubated for 5-7 hrs. often exhibited increased spike-to-EPSP ratios (and sometimes exhibited multiple spikes), in a pattern similar to that seen with high K^{+}. However, this effect was partially inhibited by incubation in low K^{+}. Conceivably, then, slices may increase in excitability over time because of K^{+} accumulation in extracellular spaces. (Supported by AG 01737).

259.11 AN AGAROSE EMBEDDING METHOD FOR INTRACELLULAR RECORDING FROM MAMMALIAN BRAIN SLICES. R.W. Snow and F.E. Dudek. Dept. Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Brain slices maintained in vitro have become popular for intracellular electrophysiological studies of membrane properties, local synaptic circuitry, and pharmacology. For many types of studies it would be advantageous to visualize neurons and microelectrode tips with better clarity than possible with the typical 300-500 µm slice viewed through a dissecting microscope. Therefore, we have developed an alternative way to mount, view, and record intracellularly from brain slices and other tissues in a chamber with good optical properties.

Rat hippocampal slices of 200 µm thickness were prepared in a cold room with a tissue chopper. The slices were incubated in a storage chamber for 1 hr at room temperature (24°-28°C) in oxygenated saline containing 1.5% Sea Prep 15/45 Agarose (FMC Corp.). When dissolved by heating to 45°C, this agarose remains liquefied at room temperature, but after gelling (below 15°C) it stays firm when heated to 37°C. Following incubation, a drop of agarose-saline containing a slice was placed in the well of a chamber. The chamber was milled from Plexiglass 9 cm x 2.5 cm x 2 mm. The central well was 1.25 cm diameter x 0.5 mm. The bottom of the well was a microscope cover glass glued to the underside of the chamber. The chamber was placed briefly on ice to gel the agarose and then was placed on the stage of a compound microscope (Aus Jena Ergeval) modified with Hoffman modulation contrast optics. Oxygenated saline at room temperature was superfused in a thin stream across the top of the slice.

Stimulation of the alveus evoked antidromic field potentials of 8-12 mV in CA1. The slices remained viable (field potential amplitude constant) for the course of each experiment (at least 2 hrs). In one case a CA1 neuron with 60 mV resting potential and 32 MΩ input impedance was held stably for nearly 2 hrs. Antidromic spikes of up to 80 mV and orthodromic EPSPs could be evoked for the duration of the impalement. Individual pyramidal neurons and granule cells could be visualized, but to date successful recordings have only been obtained from neurons too deep to be seen clearly without the intracellular injection of a fluorescent dye to increase contrast. This technique may facilitate the recording from visualized mammalian central neurons.

Although further work is required to perfect this methodology, the technique is useful now and is adaptable to other types of brain slices and other tissues such as glands.

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259.12 FIRING CHARACTERISTICS OF VENTRAL HORN NEURONS OF NORMAL AND WOBBLER MICE RECORDED IN VITRO. L.A. Mussio*, M.M. Behbehani and S.L. Pomeroy. Dept. of Physiol., U. Cincinnati Coll. Med., Cincinnati, OH 45267

The wobbler mouse develops a degenerative disease which causes cell death in the spinal cord and in several brain areas. Although this animal has been used as a model of Amyotrophic Lateral Sclerosis, no physiological data on the function of the ventral horn cells in this animal are available. This study compares the spontaneous firing rate of ventral horn neurons in wobbler and control mice. Wobbler mice were obtained by breeding heterozygote mice. The animals used in the experiments showed all the symptoms of the disease, including forelimb paralysis and atrophy, a tremulous gait, small size, and eye infections. Normal three week-old Swiss Wistar mice were used as controls. The mice were anesthetized with 150 mg/kg of chloral hydrate and, following a laminectomy, a section of lumbosacral spinal cord with attached roots was removed and immersed in oxygenated Krebs-Ringer solution containing 124mM NaCl, 5mM KCl, 1.3mM $MgSO_4$, 2.5mM $CaCl_2$, 1.24mM KH_2PO_4, 26mM $NaHCO_3$ and 10mM glucose at room temperature (280-300 mosm/lit., pH 7.2-7.4). After removal of the dura and the pia under a dissecting microscope, the cord was sectioned into 450 micron slices using a McIlwain tissue chopper. The sections were then placed on a platform inside a static chamber at 37°C and were oxygenated with 95% O_2 and 5% CO_2. Single unit extracellular recordings were made from 59 cells in the normal mouse and 37 cells in the wobbler mouse. In normal animals, the mean baseline firing rate was 13.6±.46 (SEM) whereas in the wobbler mice the mean firing rate was 30.6±.6 ($t=5.2$, $p<.001$). Although the baseline firing rate of the cells in the wobbler mice was significantly higher than that in the normal animals, their spike shape and amplitude were comparable. The result of this study indicates that the cells in the ventral horn of the wobbler mice undergo changes that lead to a higher frequency of firing. It is possible that the resting membrane potential in these animals is more depolarized than that of the cells in normal animals. We are currently investigating the membrane properties of these cells using intracellular techniques.

259.13 EFFECTS OF AMINO ACIDS ON IDENTIFIED SYMPATHETIC PREGANGLIONIC NEURONS IN THE CAT SPINAL CORD SLICE. Megumu Yoshimura* and Syogoro Nishi* (SPON: R.A. North), Kurume Univ. School of Med. Kurume, 830, Japan.

In studies of the central nervous system, slice preparations provide several advantages. In particular, the extracellular environment of the neurons can be artificially controlled and chemosensitivity of single neurons can be analyzed without extrinsic interference. We have recorded intracellularly from identified sympathetic preganglionic neurons and studied their electrical properties and responses to amino acids.

After anesthesia with α-chloralose and pentobarbital, cats were maintained on positive pressure respiration. Laminectomy was made from 1st to 4th thoracic segment and thoracotomy was performed in the 2nd and 3rd intercostal space. About 2 cm of spinal cord with attached ventral root and white ramus was carefully excised. The spinal cord was placed on a plexiglass plate using a cyanoacrylate adhesive and slices of 300 to 500 μM thickness were cut using a vibratome. The slice was transferred to a recording chamber and the upper and lower surfaces of the slice were superfused with a Krebs solution equilibrated with 95% O_2 - 5% CO_2 and maintained at 37°C. Intracellular recordings were made from intermediolateral nucleus with K citrate filled electrodes with tip resistances of 100 to 140 MΩ. Some of the intermediolateral cells were activated antidromically by white ramus stimulation.

Identified sympathetic preganglionic neurons had resting membrane potentials of -50 to -65 mV and input resistances of 33 to 74 MΩ. Conduction velocities were 1.2 to 8.3 m/sec. In about 80% of the preganglionic neurons, the action potential had a long-lasting afterhyperpolarization (1-7 sec). Cells responded to focal stimulation with an orthodromic action potential or an excitatory post synaptic potential or, rarely, with an inhibitory post synaptic potential. L-glutamate (0.2 mM) or aspartate (0.2 mM) applied by superfusion caused a marked depolarization in 70% of the cells. The glutamate-induced depolarizations were associated with a decreased membrane resistance while the aspartate-induced depolarizations were associated with an increased resistance. Glycine (0.5 mM), gamma-aminobutyric acid (GABA 0.5 mM) and taurine (0.5 mM) caused slight hyperpolarizations accompanied by a decreased membrane resistance. Both GABA and glycine effectively inhibited spontaneous firing of the neurons. All the responses were unaffected by TTX.

259.14 THE EFFECT OF TEMPERATURE ON ELECTRICAL INTERACTIONS BETWEEN ANTIDROMICALLY STIMULATED FROG MOTONEURONES AND DORSAL ROOT AFFERENTS. H. Cruzblanca* and F.J. Alvarez-Leefmans (SPON: E.J. Muñoz-Martínez). Department of Neurosciences, CINVESTAV del IPN. Apartado Postal 14-740, México 14, D.F.

The effect of temperature on electrical interactions between antidromically stimulated motoneurones (MNs) and dorsal root (DR) afferents was studied in the isolated and hemisected spinal cord of the frog, superfused with Ringer in which Ca^{2+} was replaced by 2 mM Co^{2+} or Mn^{2+}. Suction electrodes were used for stimulating and/or recording from DR or ventral roots (VR) from segments VIII or IX. Antidromic field potentials (AS) were monitored from the motor nucleus with microelectrodes (2-10 MΩ) filled with the Co^{2+} or Mn^{2+} containing Ringer. At bath temperatures above 10°C, supramaximal VR stimuli elicited in the segmental DR a single brief short-latency depolarizing potential (cf. Grinell, A., J. Physiol. 210: 17, 1970) here referred to as VR-DRP I. When bath temperature was kept between 9 and 10°C a second depolarizing potential (VR-DRP II) appeared following the VR-DRP I. Amplitude and duration of the VR-DRP II and the AS increased as the bath temperature was decreased, reaching a maximum at 3°C. Between 8 and 3°C VR-DRP II amplitude increased by approximately 20%/°C. These effects were reversible. In contrast, VR-DRP I did not show substantial changes with temperature within the range of 3 to 11°C.

The stimulus intensities required for reaching maximal response amplitudes were lower for the VR-DRP I than for the VR-DRP II. Both responses were usually graded although the VR-DRP II, unlike the VR-DRP I, appeared to be composed of unitary events, probably all or none depolarizing potentials from individual DR fibres, as suggested also from intrafibre recordings. The stepped fluctuations in amplitude of VR-DRP II were also observed if the VR was stimulated supramaximally at 1 Hz and became more apparent at 8 Hz. The AS and the VR-DRP I did not show such fluctuations.

Double shocks to the VR showed facilitation of the VR-DRP II, which was maximal between 50 and 80 msec while VR-DRP I and AS remained unchanged. It is suggested that VR-DRP I and II result from electrical coupling between two sets of afferent terminals ending monosynaptically on different regions of the soma-dendritic MN membrane. VR-DRP I reflects coupling between soma-dendritic MN membrane and a relatively few and easily saturable number of the DR fibres, while VR-DRP-II results from coupling between distal MN dendrites and the bulk of the DR fibres ending monosynaptically (cf. Alvarez-Leefmans et al, J. Physiol. 292: 387, 1979). Lowering the temperature increases antidromic dendritic depolarization produced by AS. The apparent enhanced coupling is likely to result from changes in the properties of non-junctional rather than junctional membranes.

259.15 CROSS-CORRELATION OF SPIKE TRAINS: ESTIMATION OF POTENTIAL WAVEFORMS FROM THE WAITING-TIME HISTOGRAM, C. K. Knox, M. Passatore*, R. E. Poppele and C. A. Terzuolo. Lab. of Neurophysiol., Dept. of Physiol., University of Minn., Minneapolis, MN 55455.

By cross-correlating pre- and postsynaptic spike trains, X(t) and Y(t), it is possible to estimate the time course of the subthreshold potential changes relating the two. Theoretically, the cross-correlation function $R_{XY}(\tau)$, which describes the probability of observing an output spike at a time τ before or after an arbitrary input spike, can be shown to be related to a convolution integral involving a 'waiting-time' probability, $f_W(\tau)$, and an autocorrelation function, $R_{YY}(\tau|W)$, of the output spike train. The waiting-time probability describes the chances of observing the first succeeding output spike after any arbitrary input spike, and is related to the waveform of the subthreshold potential change resulting from the temporal summation of one, two, three, etc. synaptic potentials:

$$f_W(\tau)/[1 - F_W(\tau)] = g_{\theta 1}(\tau) + g_{\theta 2}(\tau) + g_{\theta 3}(\tau) + ..$$

$F_W(\tau)$ is the time-integral of $f_W(\tau)$, while the terms $g_{\theta 1}$, $g_{\theta 2}$, etc. involve the time-derivative and successive time-integrals of the time-derivative of the synaptic potential waveform. Algorithms for obtaining an estimate of the synaptic potential waveform from the waiting-time histogram based on this relationship will be described.

Corroborating experimental data obtained from cat spinal motor neurons have also been obtained. In these experiments, single motor neurons were impaled and current pulses triggered by a Poisson noise source were passed into them while recording the evoked spike activity. Waiting-time histograms were constructed between the Poisson pulse train and the evoked spikes. From the intracellular potential records theoretical solutions for the waiting-time probability were derived and found to correspond closely to the measured histograms.

260 SYMPOSIUM. FUNCTIONS OF MULTIPLE TRANSMITTERS IN NEURONS. E. Mayeri, Univ. of California, San Francisco and California Coll. of Podiatric Medicine (Chairman); E.J. Furshpan, Harvard Medical School; J.M. Lundberg*, Karolinska Inst., Stockholm; Y.N. Jan, Univ. of California, San Francisco; R. Scheller*, Columbia Univ. CPS.

Recent work indicates that individual neurons may, in certain cases, utilize two or more substances as transmitters. Speakers will present data on such multitransmitter neurons from studies on several experimental preparations. The studies use combined morphological, electrophysiological, biochemical and even molecular genetic techniques to establish the existence of multiple transmitters and to uncover novel aspects of transmitter function. E.J. Furshpan will describe studies on the transmitter repertoire of single sympathetic neurons grown in culture with cardiac myocytes. The transmitters involved are acetylcholine, norepinephrine and a purine. Jan Lundberg will discuss the functional significance of coexisting peptides and amines in peripheral autonomic neurons. In these neurons a vasoactive intestinal polypeptide-like substance coexists with acetylcholine, and an avian pancreatic polypeptide-like substance coexists with norepinephrine. Yuh Nung Jan will describe studies on the coexistence and corelease of acetylcholine and a peptide from the same preganglionic fibers in frog sympathetic ganglia. The peptide resembles leutinizing hormone-releasing hormone. Earl Mayeri will describe chemical and physiological studies of three peptides that are candidate transmitters for mediating various actions of bag cell neurons in the abdominal ganglion of the marine mollusk Aplysia. Richard Scheller will then present related studies involving recombinant DNA techniques which show that the three peptide transmitter candidates for bag cell actions are represented on a single bag cell gene. The bag cell gene is one of a multigene family encoding polyproteins which are involved in the regulation of egg-laying behavior in Aplysia.

261 THE NEUROBIOLOGY OF FEEDING BEHAVIOR. E. Stellar and A. N. Epstein. Inst. of Neurological Sciences, Univ. of Pennsylvania, Phila., PA. 19104.

This symposium reviews the highlights of recent research on the neurobiology of feeding behavior of mammals. It will begin with a discussion of the development of ingestive behavior in the infant rat by Hall. Two papers will be concerned with brain-stem mechanisms controlling feeding behavior. One by Grill will consider neural controls caudal to the forebrain as seen in the decerebrate rat fed through an oral fistula. The second by Miselis will describe food intake and body weights of rats with lesions of the area postrema and surrounding caudal medial nucleus of the solitary tract that effectively eliminate the afferent input of the vagus nerve. Turning to forebrain mechanisms, Rolls will discuss the neuronal activity that is produced in the monkey in response to food and non-food stimuli during hunger and satiation. Hoebel will extend the discussion to the role of catecholamines and peptides in feeding and the reward of self-stimulation and self-injection. The last paper by Smith will concern postprandial satiety and the role of cholecystokinin and the vagus nerve. Each of these reviews represents a facet of the neurobiological mechanism that underlies hunger and satiety, the regulation of caloric intake, and food preferences and aversions, as they are given in the "hard-wiring" of the animal and as they are modified by experience.

262.1 EXTRINSIC CELLULAR FACTORS ARE CONTRIBUTING TO THE GOLDFISH OPTIC NERVE REGENERATION. M. Schwartz and D. Neuman*. Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel 76100

The goldfish visual system represents a good model to investigate the mechanism underlying central nervous system (CNS) regeneration, mainly in an attempt to localize the mammalian CNS deficiency.

It appeared that the low regenerative capacity in mammals may evolve from environmental factors among which are proliferating non-neuronal cells. We have recently adapted the used of X-irradiation for limiting proliferation of cells associated with the regenerative nerve in order to minimize their contribution to the process of regeneration. A promoting effect on some regenerating parameters was observed when the goldfish visual pathway was exposed to X-irradiation at the early stage of regeneration. On the other hand X-irradiation at a later stage of regeneration when the regenerating fibers reach the vicinity of the tectum had an inhibitory effect. The inhibitory effect was monitored by several procedures including the injection of ^{3}H-proline to the regenerating eye and determining the accumulation of ^{3}H-labeled material in the contralateral tectum.

We attribute the promoting and the inhibitory effects of X-irradiation on regeneration to the selective block of cell proliferation at two distinct zones; the former relates to the zone of the lesion while the latter to all the area distal to the lesion, including the tectum. The profile of labeled proteins in the regenerating retina measured by one and two-dimensional gel electrophoresis did not seem to be affected by the various modulating conditions. Furthermore, histological sections through the retina stained with haematoxiline eosin did not reveal any changes due to the irradiation. Therefore, it is suggested that the X-irradiation modulates the extent of regeneration while it does not affect qualitatively intrinsic parameters related to the retinal ability to support optic nerve regeneration.

Further studies are required to elucidate the type of cells which contribute to regeneration ability and their mode of action.

262.2 ENHANCED LABELING OF MICROTUBULE-ASSOCIATED TAU FACTORS AND ß-TUBULIN IN GOLDFISH RETINA DURING OPTIC NERVE REGENERATION. M. Schwartz, T. Scherson*, D. Neuman*, B. W. Agranoff and U. Z. Littauer* (SPON: I. Ginzburg). Dept. of Neurobiology, Weizmann Institute of Science, Rehovot 76100 Israel and Neuroscience Lab, University of Michigan, Ann Arbor, MI 48109.

The ability of nerve cells to undergo regeneration appears to be determined by intrinsic properties, as well as environmental factors. The modulation of the synthesis of several proteins has been shown to be involved in this process. Following axotomy of the goldfish optic nerve there is enhanced labeling of tubulin, the protein subunit of microtubules. In the present study we show an enhancement in [^{35}S]methionine labeling of tubulin which is mainly in the ß-tubulin subunit. Ten days following crush of the right optic nerve the retina from the two sides were excised and pulse-labeled for 1 hr with 5 µCi of [^{35}S]methionine in Dunlop buffer. The labeled proteins were examined by one- and two-dimensional gel electrophoresis and by immunoprecipitation using antibodies directed against chicken tubulin. In addition, microtuble-associated proteins (MAPs) were also purified from retina of the post-crush side and the contralateral side by using a phosphocellulose column. A selective increase in labeling of two MAPs was found in the post-crush retina. The two MAP species coassembled with tubulin and comigrated with brain TAU factors. The increase in the synthesis of two TAU factors and of ß-tubulin in the post-crush retina suggest that these proteins play a regulatory role in optic nerve regeneration by participating in the formation of the new axonal matrix. The interrelationship between TAU factors and tubulin is not yet known. Thus, it is possible that either the synthesis of each species modulates the expression of the other, or that the syntheses of TAU and tubulin are independent. (Supported by Muscular Dystrophy Association Grants to UZL and MS.)

262.3 CALCIUM SPIKES IN REGENERATING GIANT AXONS OF THE LAMPREY SPINAL CORD. B. A. MacVicar and R. Llinas. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York 10016.

Intracellular Ca is believed to play a critical role in regeneration and growth of neurons. The influx of Ca has been reported in developing Purkinje cell dendrites (Llinas & Sugimori, Prog. Brain Res., 51:323, 1979), in regenerating axons (Meiri et al., Science, 211:709, 1981; Strichartz et al., Soc. Neurosci. Abst., 6:660, 1980) and in growth cones of neurites in culture (Grinvald & Farber, Science, 212:1164, 1981). However, the small size of growth cones in most vertebrate neurons prevents rigorous analysis of ionic requirements. For this reason we have examined the Ca component of the action potential in giant reticulospinal axons during regeneration in the lamprey. The large size of the growth cone in the axons of this primitive vertebrate permits precise determination and localization of Ca spikes.

Experiments were performed on larva (8-14 cm) of the lamprey, Petromyzon marinus. Animals were initially anesthetized in Tricaine and their spinal cords severed between the 2nd and 3rd gill openings. Six to ten days later the brain and spinal cord were removed and maintained in vitro at 15°C while perfused at 1-2 ml/min. When the preparation was transilluminated, giant axons and their growth cones could be observed and directly impaled with microelectrodes containing 3M K-acetate or 5% Lucifer yellow. In some cases Lucifer yellow was injected to verify the site of impalement.

Six to ten days after the spinal cord was severed, large bulbous swellings could be observed at the end of the giant axons a few hundred micrometers proximal to the site of the cut (Selzer, J. Physiol., 277:395, 1978; Wood & Cohen, J. Neurocytol., 10:57, 1981). Action potentials were evoked by intracellular current injection or by spinal cord stimulation at areas more proximal to the brain. Bath application of tetraethylammonium (10-15 mM), 4-aminopyridine (4-6 mM) and Ba (4-6 mM) resulted in a greatly prolonged action potential. The faster initial phase was blocked by tetrodotoxin (1 x 10^{-5}M) and is presumably due to Na influx. However, the slower prolonged plateau phase was resistant to tetrodotoxin but was blocked by Co (4-6 mM). This component of the action potential is Ca-dependent. Axons from control animals did not show Ca-dependent spikes under similar pharmacological conditions. Therefore, these results support the view that Ca currents are associated with and are important in the growth process. Supported by grant NS13742 and by an NSERC postdoctoral fellowship to B.A.M.

262.4 EFFECTS OF SPINAL TRANSECTION ON BRAIN CELL BODIES AND PROXIMAL AND DISTAL SPINAL AXONAL SEGMENTS OF THE LAMPREY. R.D. Clark and W.O. Wickelgren. Dept. Physiol., U.Colo.Med.Sch.,Denver CO 80262

The larval lamprey shows partial spinal cord regeneration and resumes coordinated swimming in caudal parts after spinal transection. The present study characterized changes in lamprey brain cell bodies and spinal axon segments of large reticulospinal neurons (Müller cells) after transection/axotomy. Larval lampreys underwent spinal transection under anesthesia and were kept at 10°C. Isolated brain-spinal cord preparations were used for electrophysiological testing employing standard intracellular techniques and for anatomical viewing using injected Lucifer Yellow.

Cell bodies. A comparison of electrophysiological parameters of control (5 animals) and axotomized (7 animals) Müller cell bodies 18-41 days after axotomy yielded the following: significantly ($p<0.02$) increased in axotomized group: total spike amplitude, overshoot, undershoot, dV/dt; significantly decreased in axotomized group: conduction velocity; no significant change: resting potential, input resistance, rheobase, time constant, $\frac{1}{2}$ amplitude spike duration. Some of the changes may reflect biosynthetic events occurring after axotomy. There appeared to be no change in cell body Ca conductance in the time period 18-41 days. Dye injections in a range from 11 to 90 days postoperative revealed little change in the cell bodies and dendritic trees of axotomized Müller cells, except for slight thickening of 1° dendrites.

Proximal axons. For the first 24 hours after transection the cut ends of Müller axons did not appear to seal; resting potential increased from the cut end proximally as fit by $E_m=E_{m\infty}(1-e^{-x/\lambda})$, where E_m=resting potential at distance x from the cut end and λ = space constant (1.0-1.5 mm). From 3-24 days postoperative, proximal stump axons frequently formed large bulbous processes (end-bulbs) 2-3 mm from the cut end. Resting and action potentials of end-bulbs and rostral axons were generally normal, although some were low, perhaps due to electrode damage.

Distal axons. Immediately after axotomy the resting potential increased from the cut end in a manner similar to proximal-stump axons. Continuity of axons up to the lesion was observed up to ten days postoperative, without obvious end-bulb formation. Beginning several days after the lesion, the resting and action potentials for distal axons at all postoperative times were generally low. Two preparations were surveyed at 94 and 105 days, and, remarkably, all distal axons had at least some resting potential and small action potentials. One axon (at 94 days) had completely normal properties. All distal Müller axons were grossly swollen at these late dates. There was no electrical reconnection to the spinal cord anterior to the lesion. Such long survival of distal axons indicates substantial metabolic independence from the soma and nucleus. Research supported by NIH Grant No. NS 09661.

262.5 AXONAL REGENERATION IN TRANSECTED SPINAL CORD OF LARVAL LAMPREY. H.S. Yin and M.E. Selzer. Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Spinal cords of sea lamprey larvae were transected either rostrally at the level of the last gill or caudally at the level of the cloaca. Regeneration of caudally projecting large reticulospinal axons (RAs) and rostrally projecting giant interneurons (GIs) and dorsal cells (DCs) was studied using intracellular injections of HRP in vitro following various survival times. Individual regenerated neurons or axons were examined in wholemount preparations of spinal cord. After transection, axons retracted up to 2 mm and then sent out 1-7 banches which grew back toward the scar.

In rostrally transected spinal cords, RAs grew past the scar by approximately 40 days post-transection and reached peak distances of up to 5 mm by 50-100 days. Thereafter, the mean number of branches per axon decreased (from 3.2∓0.7 SE to 1.3∓0.3) as did the average distance of regeneration per branch (from 828μ∓275 to 109μ∓664 beyond the scar). In caudally transected spinal cords, only 3 of 28 branches in 16 injected axons regenerated up to the scar and only one had grown 38μ beyond the transection. By regression analysis, it would be predicted that the average branch of caudally transected RAs would reach the scar only after 183 days.

After 40 days of recovery, 59% of rostrally transected RAs had at least one branch which regenerated into or beyond the scar. For caudally transected axons, only 23% did so. For GIs and DCs, whose axons were transected within 3.5 mm of their cell bodies, the corresponding regeneration rates were 75% and 53% respectively. The mean distance of regeneration of branches of rostrally transected RAs after 40 days was 420μ∓118, whereas for GIs it was 345μ∓214 and for DCs -77μ∓97 (77μ before the midpoint of the scar). The lower distance of regeneration for DCs is probably real and not an artifact of reduced visibility of, or HRP transport in, smaller axons, since distance of regeneration was not positively correlated with parent axon diameter for RAs or DCs.

It is concluded that both rostrally and caudally projecting axons of identified types of neurons in lamprey larvae are capable of regeneration across a transection. The rate and degree of regeneration is probably greater the closer the axotomy is to the cell body. For axons transected close to the cell body, distance of regeneration does not increase after 50-100 days. Indeed the present data suggests that after this time there may be some neurite retraction despite continued behavioral recovery. NIH Grants NS14837, NS14257, RR05415.

262.6 REGENERATION OF CNS AXONS AFTER CRUSH INJURY IN PNS GRAFTS. S. David* and A.J. Aguayo (SPON: Patricia A. Walicke). Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Canada.

In previous experiments we have shown that spinal and medullary neurons are capable of extensive axonal elongation through PNS grafts (Science 214: 931, 1981). In these experiments it was not known whether the axonal growth resulted from elongation of injured CNS axons or from collaterals of uninjured neighbouring neurons. We now present evidence which suggests that axons from injured CNS neurons are indeed capable of regeneration.

In 8-10 week old Sprague-Dawley rats, an autologous sciatic nerve segment 3.5 - 4 cms long, was used to connect the medulla oblongata to the lower cervical spinal cord. After 6-42 weeks the grafts were crushed approximately 1 cm from both their rostral and caudal insertions into the CNS.

In one group of rats, the source of axons reinnervating the crushed grafts was examined by retrograde transport of HRP. From 4 to 11 weeks after crush, the grafts were transected at the mid-point along its length and HRP (20%) applied to the cut ends. In the second group, the cells innervating the grafts at the time of crush were labelled using Fast Blue (FB,3%), a retrogradely transported fluorescent dye. After 2 weeks, reinnervation of the grafts was examined using a second fluorescent marker, Nuclear Yellow (NY, 1%).

Labelled neurons were found in the medulla and spinal cord of both groups. The number and distribution of the HRP labelled neurons were comparable to that found in control grafted animals in which the grafts were not crushed. After using fluorescent dyes less than 15% of all labelled neurons (FB,NY and FB + NY) were double labelled with FB + NY while less than 0.5% were single labelled with NY. This suggests that the majority of the cells which reinnervate the grafts had also innervated them prior to the crush.

These results indicate that nerve cells in the CNS are not only capable of extensive axonal elongation through PNS transplants, but that such regrowth can arise from injured central neurons.

262.7 ADULT MAMMALIAN SPINAL CORD REGENERATION BY FETAL SPINAL IMPLANTS. U. Patel* and J.J. Bernstein. Department of Physiology, The George Washington University, School of Medicine, Washington, D.C. and Veterans Administration Medical Center, Washington, D.C. 20422.

For an implanted fetal spinal cord to act as a successful regenerate in an adult host, it must survive, grow, differentiate, and acquire the required connectivity. The pre-requisites for successful regeneration reside in the inherent capacities of the implant and its placement, to ensure the required contacts and the vascularization of the implant.

Fetal spinal cords (E11 to E15 post gestation) were implanted subpially into adult Sprague-Dawley male rat (250-350 gms) spinal cords, and were evaluated for growth and differentation patterns in the implants 7-30 days later. The implants were 0.15-2.5 mm at T5-T7. Over 50% of the implants survived in 30 recipients. The viability of E11 was 30% greater than those from E15 and the implants were more robust in the 21-30 day post implantation group, as compared to those in the 7-14 day group. E11 spinal implants measured about five fold linearly and 125 fold volumetrically, while E15 increased two and eight fold respectively. In addition, younger spinal implants had increased numbers of capillaries in the implants (from the host) and a greater degree of cell maturation. The differentiating cell organelles from the host in the implant were compared with those of the embryonic (E11-E15) cells over days post operative of implant and were compared with control pup liters The organized aggregation of chromatin material in the nucleus, a definite nuclear envelope with organized nucleolus, and nuclear indentations were indication of maturation. Implanted fetal spinal cord cells showed increased smooth endoplasmic reticulum, and mitochondria with more cristae over days post operative and days gestation of implant. The connectivity of the implants will be followed with wheat germ agglutinin-horseradish peroxidase and the rate of division with autoradiography and (3H) thymidine grain dilution.

262.8 MORPHOLOGY OF REGENERATING SPINAL CORD AXONS INTO A COLLAGEN MATRIX BRIDGE. J. C. de la Torre and P. K. Hill. Northwestern Univ. Med. Sch. and Eastern Virginia Med. Sch.

A morphologic study using light, histofluorescence and electron microscopy was undertaken to evaluate regeneration of spinal cord axons following transection. Long-Evans hooded rats 350-450g were anesthetized and subjected to a 200g/cm accelerating force injury to T_{10}. Ten days later, all rats underwent total spinal cord transection at T_{10}. Rats were randomized into two groups: Group I control had the severed spinal cord re-aligned end-to-end in close juxtaposition; Group II had 3 mm of spinal cord tissue trimmed from the proximo-distal stumps. This tissue was replaced with a cell-free bovine collagen matrix ((CM)) (de la Torre et al, Neurology 32: A213, '82) which acted as a bridge between the transected cord ends. After 90 days, all rats were sacrificed and the spinal cord tissue was processed for electron or light microscopy.

Electron microscopic examination of the CM and controls showed many myelinated and unmyelinated axons in the proximo-distal tissue of both groups and within the CM. Myelinated axons were seen surrounded by Schwann cells. Silver stains showed many blood vessels, neurofibrils and fibroblasts entering the CM from both the proximal and distal interfaces with the CM but no neurofibrils and few blood vessels were observed entering the scar fibers in controls.

SPG histofluorescence analysis showed many catecholamine-containing varicosities (CCV) within the CM and at the proximo-distal junction with the CM. CCV were noted in cord tissue distal to the lesion. CCV was observed only in the proximal cord stump in controls; no CCV were seen within the scar fibers or in the distal cord tissue. The invasion of myelinated axons by Schwann cells in both groups of rats suggests two or both possibilities: a) the myelinated axons are entering the cord tissue from the periphery, for example, dorsal root ganglia, b) central myelinated axons are being repaired by migrating Schwann cells from nearby root entry zones (Harrison et al Proc. Aust. Assoc. Neurol. 12:117, '75).

The results indicate that spinal cord axons and blood vessels appear to grow well into a CM used to bridge transected proximo-distal cord stumps. Few blood vessels and no axons with CCV were seen within the scar tissue or distal cord stump in controls.

262.9 FETAL BRAINSTEM MONOAMINERGIC NUCLEI TRANSPLANTED INTO THE TRANSECTED SPINAL CORD OF THE ADULT RAT. J.W. Commissiong. Dept. of Physiology, McGill University, Montreal, Quebec, Canada.

From the results of neurophysiological experiments, it is predicted that a spinal generator for locomotion exists entirely within the spinal cord (Grillner, S. Physiol. Rev. 55, 247-304, 1975). The generator is presumably activated by several descending projections, including a noradrenergic projection. When the spinal cord is transected at the thoracic level, it is likely that the neuronal substrates of the generator at the lumbar level remain intact. When L-DOPA, the precursor of catecholamines is injected into the acutely spinalized animal, it reactivates the generator, and coordinated stepping is produced. One approach to studying this problem is to try to reinnervate the transected spinal cord with a noradrenergic innervation, and then to study what effects are produced on locomotion and associated spinal reflexes.

It has now been demonstrated that this approach is feasible. The spinal cord of young adult female rats was transected subpially at the thoracic/lumbar border using an aspiration technique to minimize bleeding. The locus coeruleus was dissected from sixteen day old fetuses and transplanted into the cavity between the cut ends of the spinal cord. The pia was sutured using microsurgical techniques, and the wound closed. Controls were operated similarly, except that no fetal tissue was implanted. Four to fourteen weeks later, the cord was examined using fluorescence microscopy to visualize catecholaminergic neurons. In eight out of twenty animals, the fetal locus coeruleus implant survived, and the neurons showed varying degrees of axonal growth. In the best cases, the axons extended for 2-8 mm from the cell bodies. In two cases axonal proliferation was restricted to the nucleus itself. The most interesting observation was of an intense proliferation of the adjacent, cut, rostral catecholaminergic axons. The nature of this phenomenon is such that it must be attributed to an effect produced by the implant itself. It is therefore concluded, tentatively, that the developing locus coeruleus neurons produce a substance that is capable of stimulating the proliferation of damaged adult catecholaminergic axons. The technique has been extended to implanting the solid fetal nucleus into the intumescence of the spinal cord using a micropipette. Additionally, a cell suspension has been prepared and the cells injected into the cord using a 50 µl Hamilton syringe. Neurophysiological experiments will be performed on these animals to test the effect of the implant on spinal reflexes normally thought to be regulated by descending catecholaminergic fibres.

262.10 REGENERATION OF SENSORY-MOTOR SYNAPSES IN THE SPINAL CORD. E. Frank, S. Jhaveri* and D.W.Y. Sah*. Department of Neurobiology, Harvard Medical School, Boston MA 02115.

Little is known about the specificity of synaptic reconnection after injuries in the vertebrate CNS. We have studied the formation of sensory-motor synapses in the spinal cord of bullfrogs after dorsal root section. The 2nd dorsal root (DR2), which contains all the sensory fibers that innervate the arm, was cut close to the spinal cord. The extent of regeneration was determined 2 to 12 months later. Sensory afferents were labeled with horseradish peroxidase applied to the cut end of the brachial nerve, or injected into the dorsal root. Intracellular recordings made from motoneurons in the isolated spinal cord were used to measure the monosynaptic excitatory input (EPSP's) they received from muscle sensory afferent axons.

When DR2 was cut in late-stage tadpoles (stage 18-21), the animals often regained nearly normal use of their arms after metamorphosis, and withdrew their arms in response to pinching. There was regrowth of the normal projections of sensory axons back into the spinal cord, both to the dorsal horn adjacent to the dorsal root entry zone and of the long fiber-tracts in the dorsal columns where the fibers project rostrally and caudally for several spinal segments. Sensory fibers also re-established functional connections with appropriate motoneurons. The median ratio of EPSP's from triceps muscle afferents in triceps vs. non-triceps (subscapular and pectoralis) motoneurons was 6.3 (range, 2.1 to 48.6), compared to 11.9 for normal frogs.

When DR2 was cut in post-metamorphic frogs, there was also specific synapse reformation although the behavioral recovery was small. Frogs frequently held their arms in abnormal positions and did not withdraw them in response to pinch. Sensory fibers regenerated into the spinal cord but the long fiber-tract projections of these fibers in the dorsal columns were absent. However, within 1 mm of the dorsal root entry zone, these fibers made a normal anatomical projection in the dorsal horn. Within this region, synaptic potentials were produced in motoneurons by stimulation of muscle sensory afferents. The median ratio of triceps sensory EPSP's in triceps vs. non-triceps motoneurons was 7.8 (range, 1.9 to 11.3) All these motoneurons are located near each other and within the region of regenerated sensory arborization.

These results show that specific, functionally appropriate synapses can be re-established in the vertebrate CNS following injury. In adult frogs, there was no regrowth of long-fiber tracts, just as in the adult, mammalian CNS. Nevertheless, within the region of local regeneration, highly specific synaptic connections were formed.

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262.11 LESIONING AND RECOVERY OF NIGRO-STRIATAL DOPAMINERGIC NEURONS: EFFECT OF GM_1 GANGLIOSIDE TREATMENT. G. Toffano, G. Savoini,* F. Moroni, M.G. Lombardi* and L.F. Agnati*. Dept. of Biochemistry, Fidia Research Laboratories, 35031 Abano Terme, Italy; Dept. of Pharmacology, University of Florence, 50134 Florence, Italy; Dept. of Human Physiology, University of Modena, 41100 Modena, Italy.

Little is known about the precise mechanism governing regenerative growth in the central monoaminergic neurons. In searching for factors which can influence a trophic neurite growth response particular attention has been devoted to gangliosides. Gangliosides have been shown to stimulate morphological and biochemical differentiation of neuronal clonal and primary cell cultures. Moreover, ganglioside administration results in a facilitation of axonal sprouting both in CNS and PNS. In the present study we have investigated the effect of monosialoganglioside GM_1 on the recovery of nigro-striatal dopaminergic parameters after unilateral hemitransection (U.H.). Comparison of the time course of changes in TH activity in the lesioned striatum after U.H. of animals treated with 30 mg/kg i.p. GM_1 to that of rats treated with saline shows a marked influence of GM_1 treatment on the recovery of TH activity. In rats treated with GM_1 a significant ($p < 0.01$) increase of Vmax for TH was observed on day 14 (GM_1 treated group: Vmax of unlesioned side 20.21 $\pm$ 0.93, Vmax of lesioned side 17.90 $\pm$ 1.01; saline treated group: Vmax of unlesioned side 21.06 $\pm$ 1.03, Vmax of lesioned side 9.10 $\pm$ 0.92 nmole $CO_2/h^{-1}/mg^{-1}$ prot) which persisted until the end of the observation, i.e. 76 days. The effect of GM_1 is dose-dependent. A significant increase of TH-related immunofluorescence and HVA content was also determined in the striatum ipsilateral to the lesion in rats treated with GM_1. In GM_1 treated group the sensitivity to apomorphine (turning behavior) was significantly ($p < 0.01$) reduced in comparison to saline treated group. The increase of TH activity, HVA content, TH-related immunofluorescence detected in the striatum ipsilateral to the lesion and the decreased sensitivity of lesioned rats to apomorphine after GM_1 treatment, are compatible with the interpretation that a functional dopaminergic reinnervation of the striatum is facilitated by GM_1 treatment after hemitransection. The mechanism by which GM_1 elicits the above effect and the source of regrowing dopaminergic terminals in the striatum are still under investigation.

262.12 SUBSTANCE P STIMULATES REGENERATION OF THE CATECHOLAMINE FIBERS IN CAT NEOCORTEX. K. Nakai and T. Kasamatsu. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125, U.S.A.

Using glyoxylic acid (GA)-induced fluorescence histochemistry and a biochemical assay for endogenous catecholamines (CAs), we have shown quick regrowth of the central CA fibers in kitten visual cortex in which the CA terminals were priorly destroyed by the local perfusion of 6-hydroxydopamine (6-OHDA) (Nakai et al., 1981). This previous result may, at least in part, explain the observed incomplete blockade of the expected shift of ocular dominance in the monocularly deprived and 6-OHDA-treated visual cortex (Kasamatsu and Pettigrew, 1979), and the incomplete suppression by 6-OHDA of cortical cells' recovery from the effects of prior monocular deprivation (Kasamatsu et al., 1981).

In the present study we sought for factors which may promote this regrowth of CA fibers in kitten visual cortex. In light of a recent finding that substance P (SP), which is widely distributed in the mammalian central nervous system (Nicoll et al., 1980) and known to excite physiologically norepinephrine (NE) cells in the locus coeruleus (Guyenet and Aghajanian, 1979), counteracts neurotoxicity of 6-OHDA on NE neurons in neonatal rats (Jonsson and Hallman, 1982), we examined if SP accelerates regrowth of CA fibers. In all animals the superior cervical ganglia were bilaterally resected at 4 weeks of age. First, the visual cortex of five-week-old kittens had been locally perfused with 4 mM 6-OHDA for only a week, and then they were continuously injected with 3.3 mM SP for the next week through a cannula placed in the fourth ventricle. Subsequently, animals were prepared for GA fluorescence histochemistry. In the visual cortex of such animals, we consistently observed more numbers of green fluorescent fibers and terminals than the visual cortex without the SP treatment. Many regrowing CA fibers were observed right at the edge of the primary lesion caused by the placement of cannula for 6-OHDA. However, in the kitten visual cortex treated with the direct perfusion of 0.01 mM SP following the 6-OHDA perfusion, using the same implanted cannula in the cortex, the amount of green fluorescent fibers was less than the control without such SP perfusion. A muscarinic cholinergic agent, bethanechol (40 mM), which was used in place of SP to activate NE cells in the locus coeruleus, did not accelerate the regrowth of CA fibers. Furthermore, this enhanced regrowth of cortical CA fibers specific to SP administered intraventricularly was not necessarily accompanied by a notable change in β-adrenoreceptor binding sites in the visual cortex, as visualized autoradiographically by means of ^{3}H-dihydroalprenolol (Palacios and Kuhar, 1980).

The present results suggest that a reaction of NE cells specific to exogenous SP may be primarily responsible for the enhanced regrowth of partially damaged CA fibers at their distal end. (Supported by a USPHS grant EY 03409 to T. K. and a Del E. Webb Fellowship to K. N.)

263.1 SEX AND HANDEDNESS DIFFERENCES IN INTERHEMISPHERIC CORRELATIONS OF HUMAN REGIONAL CEREBRAL BLOOD FLOW (rCBF). S.Warach, R.C.Gur*, B.E.Skolnick*, R.E.Gur*, W.D.Obrist*, H.I.Hurtig*, H.I.Goldberg*, D.Younkin* and M.Reivich. Cerebrovascular Research Center of the Dept. Neurology, Univ. Pennsylvania, Phila.,PA; Dept. Psychology and Neuroscience Program, Michigan State Univ., E.Lansing, MI; and Dept. Neurology, Graduate Hospital, Phila., PA.

Cerebral blood flow is highly coupled to metabolic rate in normal brains and is therefore postulated to be an index of neuronal activity. A previous study demonstrated differential effects of sex, handedness, and task on hemispheric rCBF. This report addresses the question of sex and handedness differences in rCBF correlations for homologous regions of each hemisphere.

Right-handed males (RM; n=15), right-handed females (RF; n=14), left-handed males (LM; n=15), and left-handed females (LF; n=17) were studied with the 133-Xe inhalation technique while the subjects lay in resting wakefulness. Gray matter rCBF in eight pairs of homologous regions was calculated by Obrist's Initial Slope Index. Right-handers had greater positive correlation coefficients than left-handers (in 7 of 8 regions, $p<.05$) and females had greater positive correlation coefficients than males (in 8 of 8 regions, $p<.005$). Partialling out the effects of total brain flow did not change the general pattern of correlations. Handedness differences were most pronounced in the superior precentral (which includes parts of motor arm and premotor cortex). The correlation coefficient was greater for RM than for LM at $p<.005$. Sex differences were prominent in two pairs of inferior frontal detectors measuring rCBF in the vicinity of Broca's area and its right hemisphere homolog. These correlation coefficients were greater for RF than for RM at $p<.01$. The results are consistent with hypothesized sex and handedness differences in cerebral functional organization.

263.2 HEMISPHERIC ASYMMETRY IN THE CONTROL OF HEART RATE. A. D. Rosen*, R. C. Gur*, N. Sussman*[1], R. E. Gur*, H. Hurtig*[1] (SPON: A. A. Stein). Dept. of Neurology, Univ. of Pennsylvania, Sch. of Med., Philadelphia, PA 19104 and Graduate Hosp.[1]of the Univ. of Pennsylvania, Philadelphia, PA 19146.

Right hemispheric stimulation produces greater bilateral cerebral activation, as measured by electroencephalography and isotope clearance techniques. Reticular arousal, in turn, should be manifested in increased autonomic arousal. This possibility was evaluated by taking heart rate measurements in five consecutive patients who were administered injections of sodium amobarbital (125-150 mg) to each internal carotid artery (Wada test). Injections to the left and right carotids were separated by approximately one hour and were counterbalanced in order. Electrocardiogram monitoring was initiated five minutes before each injection and continued for 25 minutes. Interbeat (R-R) intervals were used to calculate heart rate. In all five cases heart rate increase following left carotid injection exceeded heart rate increase following right injection, in the first eight minutes post-injection.

The finding suggests the possibility of hemispheric asymmetry in heart rate control. The results can be interpreted as consistent with evidence for greater right hemispheric predominance in the regulation of arousal processes. Peripheral nervous system and cardiac factors are also taken into account in the interpretation.

263.3 NEUROELECTRIC CORRELATES OF ALTERNATING HEMISPHERIC DOMINANCE IN AFFECTIVE AND PHONETIC TASKS. A. C. Papanicolaou*, H. S. Levin, H. M., Eisenberg and B. Moore*. The Division of Neurosurgery, The University of Texas Medical Branch, Galveston, Texas 77550.

A probe evoked potentials (EPs) paradigm was employed to investigate predominant left and right hemisphere engagement in phonetic and prosodic processing of tape-recorded, emotionally charged conversation in an unfamiliar language, in twelve normal dextral male subjects. An irrelevant probe stimulus, a click of 85 db SPL in intensity and 999 μ sec duration was binaurally presented through headphones at a constant rate of 0.7/sec. Evoked potentials to this stimulus were recorded simultaneously from the left and right hemispheres (area T_3 and T_4 respectively) with linked ear reference. Recordings were made with the band-pass set between 1 and 30 Hz. The signals were amplified by a factor of 4×10^3, digitized and averaged using a Nicolet Med-80 system. EPs were recorded during three conditions separated by 5 minute rest intervals and presented in a different random order for each subject: (1) Control, during which the subjects attended only to the click; (2) Phonetic, where the subjects attended to the tape-recorded conversation in order to detect and count the occurrence of a syllable and (3) Prosodic, where the subjects were to attend to the prosody of speech and try to comprehend the emotions conveyed by the speakers. Ratios of the amplitudes of EPs obtained at each hemisphere during each processing condition over those obtained during the control condition were computed and the ratio scores, expressing task-specific EP attenuation relative to the control condition, were analyzed in a 2(hemispheres) x 2(tasks/control) ANOVA resulting in a significant interaction effect ($p<.0003$). Subsequent tests of simple main effects revealed that attenuation was greater in the left hemisphere during phonetic processing ($p<.03$) and in the right hemisphere during prosodic processing of the same verbal material ($p<.03$). These data support previous findings regarding left hemisphere dominance in language processing (e.g. Papanicolaou, A.C. Brain and Lang. 9:269, 1980) and constitute the first demonstration of right hemisphere dominance in the appraisal of prosodically conveyed emotions through the use of evoked brain activity.

263.4 RELATIONS AMONG MEASURES OF LOCAL CEREBRAL GLUCOSE METABOLISM IN HEALTHY ADULTS. E.J. Metter*, W.H. Riege, D.E.Kuhl, and M. E. Phelps (SPON: N.P. Rosenthal). VA Medical Center, Sepulveda and Dept. of Nuclear Med., UCLA School of Medicine, Los Angeles, CA 90024.

Previous studies using 18fluorodeoxyglucose (FDG) positron emission computed tomography (PCT) have reported normal mean and local cerebral metabolic rates of glucose (LCMRglc) in young and ageing individuals, but no analysis was made of area to area relationships, and what this might reveal regarding cerebral metabolic function. LCMRglc from 31 healthy volunteers aged 24-78 years were determined for 13 brain regions in each hemisphere and were expressed as ratios to individual mean CMRglc, to obtain comparable indices of regional variation in LCMRglc. Intercorrelations among the 26 regional metabolic ratios were accepted as significant at $p< 0.01$ ($r >.45$). From the matrix, two apparently separate functional metabolic systems were identified: (1) A superior system involving superior and middle frontal gyri, inferior parietal lobule, and occipital cortex; and (2) An inferior system involving inferior frontal, Broca's and posterior temporal regions. Positive correlations were found between the metabolic ratios of superior frontal and inferior parietal cortices ($r > .51$), and negative correlations between these two areas and occipital cortex (frontal $r > -.64$, parietal $r >-.45$). These areas did not correlate with metabolic ratios from inferior frontal or Broca's areas. By comparison, metabolic ratios of inferior frontal and Broca's regions, primarily from the left hemisphere, correlated negatively with posterior temporal areas ($r >.55$). All correlations were independent of age, except those of left and right Broca's area. Differences in metabolic relations between superior and inferior frontal regions suggest differences in functional relationships. Functional differences are supported by correlations of superior frontal glucose metabolism with memory and decision criteria in normal adults (Riege et al, this meeting), and by the correlations of inferior frontal, Broca's and posterior temporal glucose metabolism with language scores from aphasic patients (Ann.Neurol. 1982;10:102). The inferior frontal, temporal metabolic system appears to at least involve language mechanisms. The superior frontal,parietal and occipital metabolic system which includes visual cortex and frontal eye fields may rely upon connections through the superior longitudinal fasciculus and appears in part to involve visual processing and attentional mechanisms.

263.5 RELATION AMONG MEASURES OF LOCAL CEREBRAL GLUCOSE UTILIZATION AND MEMORY OR DECISION FUNCTIONS. W. H. Riege, E. J. Metter*, D. E. Kuhl, M. E. Phelps and R. A. Hawkins*. VA Medical Center Sepulveda and UCLA School of Medicine, Los Angeles, CA 90024.

Changes in memory functions have been taken to reflect changes in brain functions. However, this correlation has not sufficiently been verified. We have evaluated multivariate memory and decision functions with Signal Detections measures and regional estimates of brain metabolism with the [F-18] fluorodeoxyglucose scan (FDG) using positron emission computed tomography in 25 normal volunteers of age range 27-78 years. Local cerebral metabolic rates of glucose utilization (LCMRglu) were determined for 13 regions in both left and right hemispheres and were expressed in reference to a person's mean CMRglu. The ability to reconstruct designs from memory was negatively correlated both with LCMRglu of Caudate and Thalamic regions and with age ($r > -0.50$, $p < 0.02$) while recognizing designs was associated with right Occipital LCMRglu. A more remarkable separation was seen in the intercorrelations of LCMRglu with memory (d') and decision measures (β). Memory processing of words, story, or designs, and discriminating auditory patterns from memory were associated with LCMRglu of bilateral superior Frontal and Broca's regions ($r > 0.53$, $p < 0.01$), while measures of decision bias in recognition of both visual or auditory patterns were reliably and equally predicted ($F = 9.23$, $p < 0.004$) by LCMRglu of Temporal and of Occipital regions. However, the Temporal and Occipital LCMRglu were also negatively related with LCMRglu of left Frontal and Broca's regions, so that memory and decision processing may involve compensatory changes in anterior and posterior cerebral metabolic rates. Anterior LCMRglu may indicate how well a person remembered; in contrast, posterior LCMRglu were indices to measures of how certain a person was that he remembered correctly.

263.6 VISUAL EVOKED POTENTIALS AND POSITRON EMISSION TOMOGRAPHY: CAN THE NEURONAL POTENTIAL GENERATORS BE VISUALIZED? G. G. Celesia, R. E. Polcyn,* J. E. Holden,* R. J. Nickles,* J. S. Gatley* and R. A. Koeppe.* Lab. of Neurophysiology, Univ. of Wisconsin/William S. Middleton Memorial Veterans Hospital, Madison, WI 53705.

Visual evoked potentials (VEP) and visual evoked spectrum array (VESA) to flashes and pattern-reversal were correlated with regional cerebral blood flow (rCBF) and local cerebral glucose metabolism in four hemianopsic patients and one subject with cortical blindness. Normal VEP, topographical distribution and rCBF were noted in hemianopsic patients with macular sparing. A dissociation of topographical distribution of VEP to flashes and pattern-reversal was demonstrated in one patient with hemianopsia and macular splitting. In this case, positron emission tomography (PET) showed unilateral activation of visual areas 17, 18 and 19 of the cortex. The distribution of surface-recorded potentials reflected the complex interaction of electrical field potentials within at least three cortical areas rather than volume transmission of striate dipoles alone. VEP were preserved in a cortically blind patient. PET revealed a functioning island of striate cortex that most likely represented the generator of the VEP.

The combination of VEP and PET permits the correlation of electrophysiological events with the visualization of cortical areas presumably activated by the same visual stimulus.

263.7 A NEUROLOGIC BASIS FOR HUMAN AMNESIA: THE METABOLIC APPROACH, B.T. Volpe*, P. Herscovitch*, M. Raichle, M.S. Gazzaniga. Dept. of Neurology, Div. of Cognitive Neuroscience, Cornell University Medical College, NY, NY 10021; and Dept. of Neurology and Radiation Sciences, Washington University Medical School, St. Louis, Missouri 63110.

Neuropsychological study of the human amnesic syndromes has focused on patients with focal brain damage, H.M. and N.A.; and on patients with diffuse brain damage, alcoholic Korsakoff's syndrome. The neuropathological correlates of the behaviorally well characterized amnesic syndromes have been determined by neurosurgical observation, CT scanning, or post-mortem study. Positron Emission Tomography (PET) scans of patients with amnesia offer the advantage of measuring regional cerebral blood flow and metabolism. We used a battery of standardized neuropsychological tests and a series of cognitive experiments to characterize a patient with amnesia after hypoxic ischemic brain injury. CT scanning did not reveal a morphologic injury consistent with his amnesia. PET scanning was performed using O15 labeled radiotracers to measure regional cerebral blood flow (CBF), cerebral blood volume (CBV), and cerebral metabolism of oxygen (CMRO2). Resting state PET scans showed global decrease in CBF, CBV, and CMRO2. Additionally, there were asymmetries in flow, volume, and metabolism in the left hemisphere compared to the right hemisphere. Other relative asymmetries were noted and will be shown. (Supported by the McKnight Foundation, the Alfred P. Sloan Foundation, USPHS Grant No. NS 06833; HL 13851, and by the McDonnell Center for the Study of Higher Brain Function.)

263.8 RHEOENCEPHALOGRAPHIC MEASUREMENTS OF REGIONAL CEREBRAL BLOOD FLOW: COGNITIVE EFFECTS IN NORMALS AND SCHIZOPHRENICS. R. A. ROEMER, C. SHAGASS*, R. C. JOSIASSEN* AND J. J. STRAUMANIS* Laboratory of Psychiatric Neurophysiology, Temple University and Eastern Pennsylvania Psychiatric Institute, Philadelphia, PA

Rheoencephalograms (REG) have yielded measurements highly correlated with regional cerebral blood flow (rCBF) estimates obtained by the intracarotid XE133 method. As a completely noninvasive method, REG offers possibilities for semicontinuous studies of ongoing cognitive activity.

We report here REG effects of cognitive activation in 16 normal subjects and 16 medicated chronic schizophrenic patients. Since isotopic rCBF deviations from normal have been reported in schizophrenics, we sought to determine whether the REG method would yield similar results with cognitive engagement.

Four experimental conditions yielded REG recordings from 8 scalp regions (4 from each hemisphere). Conditions were: 1) resting, eyes closed in a dark room; 2) resting, eyes open with room lights on; 3) Seashore Rhythms test; 4) Wisconsin Card Sort. Three replicate samples in each of the conditions were taken at about 2, 4, and 6 minutes.

We used Jacquy's F index to estimate flow values from the REG. Across all conditions, the anterior regions yielded higher F index values than did the posterior scalp regions (both groups). Hemispheric differences between groups were: Schizophrenics - higher right than left F values; Controls - higher left than right values.

Conditions effects (p 0.05) by scalp quadrant (L, R, anterior -A, posterior - P) in the controls were: a) eyes open vs all others - LP lower; b) Seashore and Card Sort vs eyes open - RP lower; c) Card Sort vs eyes open and Seashore - RA higher; d) Card Sort vs eyes closed - LA and RA higher. The schizophrenics showed only one of these effects - RA higher for Card Sort vs Seashore.

Effects found in schizophrenics and not in controls included: a) eyes closed vs Seashore - higher RA; b) Card Sort vs eyes open or closed - LP lower; and c) Card Sort vs alll others - RP higher.

Patient F values differed from controls as follows: a) eyes closed or open - RA higher and b) Card Sort - LP lower and RP higher.

These results demonstrate that regional REG measures shift with cognitive activity in normals and that these shifts do not occur or differ in schizophrenic patients.

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263.9 ANATOMIC ASYMMETRY OF HUMAN INSULAR CORTEX. J. R. Ellis* and J. B. Kirkpatrick. Department of Pathology, Baylor College of Medicine, Houston, TX 77030.

Anatomically corresponding regions of the cerebral cortex possessing structural asymmetry may correlate with different functional capacities of the two sides of the brain. The insular cortex (island of Reil) of the human brain, lies buried in the depths of the Sylvian fissure covered by branches of the middle cerebral artery. It may be implicated in visceral motion and sensation. This study was conducted to describe quantitatively the anatomy of the human insular cortex, with particular attention to the differences between hemispheres. Fifteen normal human brain specimens were obtained at autopsy from patients dying from a variety of causes not due to CNS disease. Ages ranged from 28 to 73 years (median 61); 4 females, 11 males; only one non-white. Handedness is not known as yet. Fresh brain weight ranged from 1205 to 1450 gm, and no lesions were detected externally or during the dissections. After thorough fixation in formaldehyde, the insulae were uncovered by breaking away the opercula. These were photographed to compare the gyral patterns. Six mm horizontal sections were made with the assistance of a jig, laid flat and photographed. Planimetry of the enlarged photographs was performed with a Zeiss digitizing board. The length of the cortical ribbon was multiplied by section thickness to obtain the surface area.

The following results were obtained: 1) To visual inspection, each of the insulae was distinct in its anatomical appearance and gyral pattern, and pairs from the same brain possessed no additional similarities to each other than to another insula from a different brain. 2) The central sulcus was the most consistent feature, although it was lacking in one case. 3) The number of short gyri ranged from 2-4; long gyri, 1-3 per insula. 4) The mean surface area of the 30 insulae was 22.2 sq cm $\pm$ 2.9 sq cm. Pair comparisons revealed a significant asymmetry with average difference between the two sides of 1.75 sq cm $\pm$ 1.3 sq cm, $t = 4.91$, $p < 0.001$. 5) The left-sided insulae tended to have a greater surface area than the right side, although not statistically significant by matched pair t test ($t = 1.3$, $p > 0.2$). 6) The cases could be divided into two groups: A higher asymmetrical group (6 cases) had greater than 11% difference in surface area, ranging to 20%. Five of the highly asymmetrical cases had the left side larger. The other 9 cases were more nearly symmetrical, varying no more than 7%. These data suggest that some humans have relative symmetry of this visceral control region. Others, however, have significant asymmetry. The functional implications of this finding remain to be explored.

263.10 AUDITORY PERCEPTION AFTER HEMISPHERECTOMY: NONVERBAL AND VERBAL MEASURES. Terry Allard* (SPON: Shirley H. Wray). Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139

Visual, somatosensory, and motor deficits are commonly observed after cerebral hemispherectomy whereas primary auditory impairments have not been described in this population. The present study was undertaken in order to determine whether such hearing defects do occur and to examine the relationship between audition and language after hemispherectomy.

Monaural auditory function was tested in two patients with left-sided cerebral hemispherectomy for the relief of epilepsy due to early brain injury. Detection thresholds for 400-ms and 1-ms bursts of white noise were obtained with a 2-interval, 2-alternative forced-choice paradigm and an adaptive algorithm, PEST (Taylor, M.M. and Creelman, C.D., J. Acoust. Soc. Am., 41: 782, 1967). Stimulus generation, response monitoring, intensity manipulation, and feedback were controlled automatically by a PDP-12 computer. The results for 400-ms noise bursts showed that both patients had normal and symmetrical thresholds. In contrast, their thresholds for 1-ms bursts were 7-10 dB higher on the right than on the left. Normal subjects had detection thresholds at both durations that were equal bilaterally.

The nonverbal perceptual impairment following hemispherectomy was accompanied by a monaural defect in the categorical perception of a computer-synthesized speech continuum varying voice-onset time. Speech sounds ranging from /da/ to /ta/ were presented to each ear at 90 dB SPL. Labeling of speech sounds presented to the left ear of both patients was stable and showed a clear distinction between the categories /da/ and /ta/. Right-ear performance was less reliable and phonemic categories were not well defined.

In summary, the ear contralateral to hemispherectomy in both patients was relatively insensitive to acoustic transients and impaired in the perception of synthetic speech sounds. The concomitant unilateral impairment in the perception of brief sounds provides a psychoacoustic framework for understanding speech perception losses and should be extended to the study of patients with lesions of the temporal lobe.

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263.11 VISUAL ORIENTING DEFICITS IN HUMANS FOLLOWING PARIETAL LOBE LESIONS. J. A. Walker, A. Henik*, M. I. Posner, and F. J. Friedrich*. Cognitive Neuropsychology Laboratory, Good Samaritan Hospital and Medical Center, Portland, OR 97210, and Department of Psychology, University of Oregon, Eugene, OR 97403.

Patients with parietal lobe lesions in either the right or left hemisphere were studied in several tasks requiring attention to either visual field. The tasks were conducted using tachistoscopic presentation, and choice accuracy as well as reaction time was recorded, when appropriate. In a task which required shifting attention from the midline to one of the visual fields, while maintaining central fixation, patients showed significant deficits in shifting attention to the field contralateral to the lesion. However, the orienting defect could be vastly improved when attention was cued to the neglected field. The latency to respond to the target in the neglected field decreased with time following the cue. The cueing effect occurred whether a central cue (an arrow pointing left or right) or a peripheral cue (the appearance of an annulus) was used.

Tasks such as letter matching and word matching were used to demonstrate the manner in which the defect influenced performance even in foveally presented tasks. Tasks using peripheral presentation as well as foveal presentation emphasized the ipsilateral/contralateral visual field differences. These data indicate that the effects of misdirecting attention in parietal patients were similar to those found in normals, but were magnified when targets were presented in the field contralateral to the lesion.

263.12 THE EFFECT OF LOCALIZED CORTICAL LESIONS ON VISUAL CLOSURE PERFORMANCE. Richard S. Lewis. Department of Psychology and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

A dysfunction of the brain causing an inability to process discrete pieces of visual information and synthesize them into a meaningful whole has been termed simultaneous agnosia. The present study investigates this dysfunction in terms of the effect of localized cortical lesions on a test of visual closure, the Street Gestalt Completion Test (Street).

A neurologically normal, hospitalized control group was utilized to study the effect of several variables on Street performance. Age and education were found to have significant effects, whereas sex and race did not. The experimental groups were composed of right-handed patients with lesions localized completely within one of the eight cortical lobes, as judged by a neuroradiologist using computerized tomography. An analysis of variance, partialling out the effects of age and education, compared the performance of each experimental group with the control group.

A significant deficit in performance was found with lesions in the right parietal, right temporal, right frontal, as well as the left frontal lobe. These results suggest that many distinct areas of cortex are involved in visual closure and that these areas are not completely lateralized to the right hemisphere.

263.13 MEDIATING PROCESSES DURING CONCEPT FORMATION BY ADULTS WITH CEREBRAL LESIONS. K. D. Cicerone*, R. M. Lazar*, D. Penman* and W. R. Shapiro* (SPON: D. Berman). Dept. of Neurology, Memorial Sloan-Kettering Cancer Ctr. and Dept. of Psychology, Queens College, CUNY.

The role of the frontal lobes in higher intellectual function has been a recurrent theme in the history of cortical localization. Deficits in conceptual ability have been variously ascribed to hemispheric differences, or to anterior vs. posterior cerebral regions. We have examined concept formation in brain tumor patients, using Levine's (1966) procedure for the analysis of mediating hypotheses and cognitive strategies during visual discrimination learning.

Subjects were 30 patients with unilateral cerebral neoplasms: 24 gliomas, 4 solitary metastases, 2 meningiomas. Patients with pre-Rolandic lesions (n=18) attained fewer concepts and used fewer appropriate hypotheses ($p < .01$) than those with post-Rolandic lesions (n=12), although there was no difference in total hypotheses used. The impairment of the pre-Rolandic group appeared to be due to difficulty in systematically eliminating irrelevant hypotheses following negative feedback ($p < .05$). Evaluation of subjects' strategies during problem-solving showed that the pre-Rolandic group used fewer appropriate (win/stay or lose/shift) strategies than the post-Rolandic group ($p < .01$). Error analyses indicated that the pre-Rolandic group was more likely to maintain an incorrect hypothesis even after being informed that it was incorrect (lose/stay) ($p < .01$); this accounted for 44% of their errors. Post-Rolandic subjects' errors were distributed across strategies; there was a nonsignificant trend for these patients to err by shifting away from correct hypotheses. The findings provide new evidence for perseverative tendencies in patients with frontal lesions.

There were no differences as a function of lesion laterality on any of the above measures (n=15 in both left and right group). Differences obtained between the pre- and post-Rolandic groups were not accounted for by differences in demographic variables (age, education) or by clinical or psychometric evidence of language disorder. Dimensional analyses suggested that the pre-Rolandic group (and especially those subjects with the greatest perseverative tendency) attended to fewer dimensions of the informing stimuli. Their deficit, however, could not be explained on the basis of either specific stimulus-preferences or more rapid memory decay. Results are interpreted as a reduced ability of subjects with anterior lesions to sample and/or integrate environmental cues which control behavior.

Ref.: Levine, M., J. Exp. Psychol., 71:331, 1966

264.1 SUBSTANTIA NIGRA DOPAMINE NEURONS: RELATIONSHIP BETWEEN CELL FIRING PROPERTIES, ANATOMICAL LOCALIZATION, AND AUTORECEPTOR SENSITIVITY. P. Shepard* and D.C. German. (SPON: L. Hersh). Depts. of Physiol. and Psychiat., U. of Texas Health Sci. Cntr., Dallas, TX. 75235.

Dopamine (DA)-containing neurons in the substantia nigra (SN) possess autoreceptors. Activation of these receptors by low systemic doses of the DA agonist, apomorphine (APO), decreases DA neuronal impulse flow. The present experiment was undertaken to examine the firing characteristics of DA neurons throughout the SN, and neuronal response properties following administration of APO.

Male Sprague-Dawley rats (200-300 g) were anesthetized with chloral hydrate. Single units were recorded with metal microelectrodes and recording sites marked with microlesions. Recordings were made between stereotaxic coordinates A1200-A2300 and Lat 1000-2500 according to the Konig & Klippel atlas. One cell was recorded in each animal and each cell was tested with i.v. injections of 5 µg/kg APO followed by 0.5 mg/kg haloperidol (HALO). In some experiments two microelectrodes were used to simultaneously record from DA cells in rostral and caudal SN loci. Cell response properties were measured in terms of (a) firing rate/10 sec., (b) interspike interval (ISI), and (c) spike height.

Cells were grouped into two categories according to their sensitivity to APO: S cells were decreased > 50% (75 ± 4%) and I cells were decreased < 50% (32 ± 2%). I cells (n = 20) were located caudally (A1200-A1600) and rostrally (A1800-A2300), whereas S cells (n = 11) were only located rostrally. The baseline firing rates of S cells were lower than I cells (2.9 ± .43 Hz vs. 4.4 ± .37 Hz, $p < .02$). The firing rates of both groups of cells were linearly related to the post-APO firing rates ($r > .90$, $p < .001$) and the slopes of the regression lines differed ($p < .001$). These data indicate that each group of cells was decreased a constant amount (number of Hz) by APO. The S cells fired with a more variable ISI than the I cells ($p < .01$), and for I and S cells together the firing rate was inversely correlated with ISI variance ($r = -.67$, $p < .001$). Spike height analysis indicated that APO increased and HALO decreased spike amplitude. Finally, evidence for the existence of S and I type DA neurons was also obtained from simultaneous recordings at rostral and caudal SN loci and cross-correlation analysis indicated that some cells fired with a high degree of synchrony.

These data indicate that I and S type nigral DA neurons have different (a) anatomical localizations, (b) electrophysiological "signatures", and (c) autoreceptor properties.

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264.2 INACTIVATION OF DOPAMINE-CONTAINING NEURONS BY CHRONIC NEUROLEPTIC ADMINISTRATION. F.J. White and R.Y. Wang. St. Louis Univ. Sch. Med., Dept. of Pharmacology, St. Louis, MO 63104.

It has been reported previously (Bunney and Grace, Life Sci. 23:1715, 1978) that chronic haloperidol (CHAL) treatment causes depolarization inactivation of dopamine (DA) containing neurons of the rat substantia nigra pars compacta (SNC or A9). In the present experiments, the effects of CHAL treatment on DA-containing neurons of the rat ventral tegmental area (VTA of A10) were compared to the effects on A9 DA cells using extracellular single unit recording techniques. The number of spontaneously active DA cells/track was systematically counted by lowering a recording electrode 10 times through a stereotaxically defined region of the VTA and SNC in groups of rats treated either acutely or chronically for one to eight weeks with HAL (0.5 mg/kg/day). Acute HAL produced a 25% increase in the number of active DA cells/track in both A10 and A9. In contrast, CHAL reduced the number of active A10 and A9 DA cells/track in a time-dependent manner. The reduction of DA cell activity occurred more rapidly and to a greater extent in A10 than in A9. Thus, the number of active A10 DA cells/track was already significantly reduced following one week of CHAL whereas the number of active A9 DA cells/track remained normal until after three weeks of CHAL. In addition, at all times tested, the percentage decline of active A10 DA cells/track (maximum reduction = 71%) was always greater than in A9 (maximum reduction = 53%). The reduction of both A9 and A10 DA cells/track approached maximum following three weeks of CHAL. Paralleling the reduced number of active DA cells during CHAL exposure was an alteration in the firing patterns of many remaining active DA cells. Rather than the slow regular discharge pattern or slow bursting pattern characteristic of normal DA cells, CHAL caused a significant proportion of the DA cells (identified by antidromic stimulation) to exhibit a highly irregular discharge pattern characterized by an unusually large number of prolonged interspike intervals. This effect also occurred sooner in A10 (following 2-3 weeks) as compared to A9 (following 4-6 weeks). Both the decrease in active DA cells and the altered firing pattern produced by CHAL treatment were reversed by intravenous injection of apomorphine (0.063 mg/kg) which hyperpolarizes and inhibits DA cells in control rats. Thus, the reduction of spontaneously active DA cells/track during CHAL treatment probably resulted from depolarization inactivation. These results suggest a new mechanism by which CHAL treatment might dampen DA neurotransmission which may help to explain the delayed onset of antischizophrenic actions and severe extrapyramidal side effects (e.g. tardive dyskinesia) which result from chronic neuroleptic treatment.

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264.3 EFFECTS OF D- AND L-AMPHETAMINE ON A10 DOPAMINE NEURONS: IONTOPHORETIC STUDIES. R.Y. Wang, Dept. of Pharmacol., St. Louis University Sch. of Med., St. Louis, MO 63104.

Browder et al. (Brain Res. 207:1981, 333) reported that l-amphetamine (l-amph) was at least as potent as d-amph in depressing presumed dopamine (DA) cells in the ventral tegmental area (VTA or A10). In contrast, I found that d-amph was 9.2 fold more potent than l-amph in producing 50% inhibition of discharge rate of antidromically identified mesolimbic and mesocortical A10 DA neurons (Brain Res. Rev. 3:1981, 153). Furthermore, my results suggest that the depressant effect of amphetamine on A10 DA neurons may be mediated by a DA dendrodendritic and/or axon collateral autoregulatory system in the VTA. To resolve the controversy of d- and l-amph effect on A10 DA cells and to determine the mode of action of amphetamine, DA, d- and l-amph were applied directly onto both DA and non-DA cells in the VTA by iontophoresis. The identification and characteristics of both DA and non-DA cells have been described previously (Wang, Brain Res. Rev. 3: 1981, 123). When applied directly to non-DA cells in the VTA, DA induced either excitation or no effect; neither d- nor l-amph had any consistent effects. By contrast, both DA and d-amph markedly suppressed firing activity of A10 DA neurons; l-amph, however, was ineffective in producing depression. The depression of firing induced by either DA or d-amph was blocked by a DA antagonist trifluoperazine. To decide whether the depressant effect of d-amph on A10 DA cells is mediated by a direct agonistic action on DA autoreceptors or indirectly by releasing DA from DA varicosities in the VTA, rats were pretreated with reserpine (5 mg/kg, ip 24 h prior to experiments) plus α-methyl-p-tyrosine (α-MT, 250 mg/kg, ip, 1 h). In these rats the depressant effect of d-amph but not that of DA was prevented. To compare possible influences of d- and l-amph on a norepinephrine (NE) system, d- and l-amph were tested on hippocampal CA2 and CA3 pyramidal cells. Both d- and l-amph were equally effective in suppressing firing activity of hippocampal pyramidal cells. In addition, pretreatment of rats with reserpine plus α-MT was unable to prevent the depressant effect of both d- and l-amph.

In conclusion, these results indicate that (1) d-amph is much more potent than l-amph in depressing A10 DA activity, (2) the depressant effect of d-amph on A10 DA neurons is indirectly mediated by releasing DA from DA varicosities in the VTA; DA would then act upon DA autoreceptors located on or near the DA soma. The results also suggest that d- and l-amph have very different effects on the NE system. D- and l-amph were equipotent in suppressing hippocampal pyramidal neurons and the effect of amphetamine on latter cells is presumably mediated by a direct agonistic action on NE receptors. (Supported by USPHS Grants MH-34424 and MH-00378).

264.4 PROGESTERONE ADMINISTRATION IN VIVO MODULATES IN VITRO DOPAMINE RELEASE FROM THE CORPUS STRIATUM OF OVARIECTOMIZED-ESTROGEN TREATED RATS. V. D. Ramirez and D. E. Dluzen. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

The effect of in vivo progesterone (P) administration on in vitro amphetamine-elicited dopamine (DA) release from the corpus striatum (CS) of ovariectomized-estrogen primed rats was examined. At 10 days post-ovariectomy female rats received 4 daily injections of estradiol benzoate (EB - 5 µg/.1 ml oil) followed by P (1.25 mg/.2 ml oil) on the fifth day at .5, 2, 4, 12 and 24 h prior to decapitation and perifusion of CS by a procedure previously described. Control groups receiving Oil+Oil, EB+Oil and Oil+P were sacrificed at the interval corresponding to the P-4h group. A total of 8 groups were included with N=4 perifusions/group. Following a one hour equilibration period, perfusate samples were collected at 4 minute intervals for 9 intervals. Upon completion of this 36 minute baseline period amphetamine (10^{-5}M), dissolved in perifusion medium (Krebs-phosphate buffer pH 7.4 containing .1% albumin, .18% glucose, .1% ascorbic acid and 3.5×10^{-4}M pargyline), was infused for one minute followed by 5 additional collection intervals. All samples were acidified to .1N with $HClO_4$ and DA determined using a radioenzymatic assay. P appears to produce a slight increase in DA baseline release (pg/mg/min) within .5h after administration ($\bar{X}$+SE = 6.9±1.7) and in the absence of EB priming when administered 4h prior to decapitation (6.0±1.0), compared to Oil+Oil (3.0±.3) and EB+Oil (3.8±.2) controls. P administration at 2 and 4 h prior to decapitation in EB primed females produced a marked increase in DA baseline release (13.3±6.1 and 17.5±6.5, respectively). Baseline DA release of P at 12 h remained elevated (9.9±4.6) and clearly returned to control values, if not inhibited, by 24 h (2.2±.5). Amphetamine infusion produced a substantial stimulation of DA release in EB primed females receiving P at .5, 2, 4 and 12 h ($\bar{X}$+SE of 5 post-stimulation intervals = 25.2±4.6, 32.2±9.9, 34.9±2.8 and 25.0±7.0, respectively). In contrast, control groups receiving Oil+Oil, EB+Oil and Oil+P demonstrated attenuated amphetamine stimulated responses (15.3±1.9, 15.6±2.3 and 12.1±1.1, respectively) and the EB+P-24h group displayed a further reduction in response to amphetamine (8.0±.9).

These results demonstrate a marked, time dependent neuromodulatory role for P administered in vivo to ovariectomized-estrogen primed rats in the spontaneous as well as in the amphetamine-stimulated release of DA from superfused CS tissue fragments, reinforcing the hypothesis that hormones play an important regulatory role in CS function. (Supported by NIH Grant HD-14625 to VDR.)

264.5 +3-PPP: EVALUATION OF EFFECTS ON PRE- AND POSTSYNAPTIC CENTRAL DA RECEPTORS. L. T. Meltzer*, J. A. Kauer*, and B. S. Bunney (SPON: S. Grant). Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510

Biochemical and behavioral studies have demonstrated that the racemic mixture of 3-(3-hydroxyphenyl)-N-n-propylpiperidine (3-PPP) has the pharmacological profile of a centrally acting selective dopamine (DA) autoreceptor agonist. We have tested this directly by utilizing extracellular single-unit recording and microiontophoretic (MI) techniques to assess the effects of 3-PPP on identified DA neurons in the zona compacta (ZC) of the substantia nigra (SN), on non-DA neurons in the zona reticulata (ZR) and on DA innervated non-DA neurons in the caudate nucleus (CN). All experiments were conducted in chloral hydrate anesthetized rats.

Two types of responses of ZC DA cells to i.v. 3-PPP were observed. In one group of cells a 50% decrease in firing rate was obtained with 32 ug/kg and total inhibition of firing was produced by a cumulative dose of 256 ug/kg. In a second group of cells, a 50% decrease was obtained with 1 mg/kg and no greater than 60% inhibition could be produced, even with cumulative doses as high as 16 mg/kg. The effects of 3-PPP on both groups of neurons were reversed by the DA antagonist, haloperidol.

In order to directly assess the effects of 3-PPP on DA cell activity, 3-PPP was applied directly onto DA cells by means of MI. DA cells were inhibited by MI DA applications. Similar to the effects of systemic 3-PPP, two responses of DA cells to MI 3-PPP were observed. In one group of cells, firing rates were decreased by MI application of 3-PPP, whereas in a second group of cells, 3-PPP had no effect on firing rate. MI administration was also found to increase the activity of non-DA ZR neurons.

The specificity of 3-PPP for pre- vs. post-synaptic DA receptors was tested by assessing the effects of MI 3-PPP on CN cells. Similar to MI DA, 3-PPP inhibited the activity of both glutamate stimulated and spontaneously active caudate neurons. Thus, racemic 3-PPP has a postsynaptic DA-like effect.

From these experiments we can conclude: (1) based on the response to racemic 3-PPP, two populations of DA cells can be identified and (2) based on its effect on non-DA ZR neurons and CN neurons, racemic 3-PPP is not selective for DA autoreceptors. Some of these results may be explained by the fact that the optical isomers of 3-PPP have opposite effects on postsynaptic DA receptors (A. Carlsson, personal communication). Both isomers are autoreceptor agonists, but one is a postsynaptic agonist and the other is a postsynaptic antagonist. Studies of the effects of the isomers on pre- and postsynaptic DA receptors are in progress. (Supported in part by USPHS grant, MH-25642, MH-14276, USPHS fellowships MH-08761 and NS-07136 and the State of Connecticut.)

264.6 THE ROLE OF NOREPINEPHRINE AS A VASOMOTOR HORMONE IN THE CEREBRAL AND PERIPHERAL VASCULATURE OF THE DOG. J.A. Louie* and D.D. Gilboe, Departments of Neurosurgery and Physiology, Univ. of Wisconsin, Madison, WI 53706.

The roles of epinephrine as a hormone and norepinephrine (NE) as a neurotransmitter are well documented. However, NE's hormonal function remains enigmatic. We investigated the vasomotor effects of circulating NE in two functionally distinct vascular beds. We measured changes in arterial pressure during intra-arterial infusions of NE in 12 isolated canine brains, perfused through the carotid arteries, and in five hind limbs perfused through the external iliac artery.

The response, the percent change in initial resistance, and the variable, effective plasma NE concentration, were calculated. These data were fitted directly to rectangular hyperbolae. In the cerebral vasculature the maximum percent change in initial resistance, maximum $\%R_i$, was 107.2 ± 9.5% with a half maximal response, ED_{50}, obtained at a NE plasma concentration of 1.31 ± 0.38 $*10^{-5}$M (N=36). In the perfused hind limb the maximum $\%R_i$ rose to 490.3 ± 30.1% (p=0.001) with an ED_{50} of 9.22 ± 2.3 $*10^{-6}$M) (N=27). Although half maximal responses were obtained at similar plasma NE concentrations (p=0.38) in both vascular beds, equipotent responses were obtained at lower NE concentrations in the peripheral vasculature.

It has been suggested that an "avid" uptake of NE by perivascular sympathetic nerves might explain the differential response of peripheral and cerebral vessels to adrenergic agonists. We have previously reported that there was no change in the maximal cerebrovascular response to NE when similar experiments were carried out 3 days after bilateral superior cervical gangliectomy (maximal $\%R_i$ =101.3 ± 5.7%). However, the ED_{50} (3.44 ± 0.74 $*10^{-6}$M) differed significantly (p=0.01) from the corresponding control. This implies that regional differences in the rate of neuronal uptake of NE cannot alone explain the different kinetic responses that we have observed in the peripheral and cerebral vasculature of the dog.

Levels of NE found in resting dogs did not constrict either experimental vascular bed. Levels of NE found in dogs during severe acidosis or hypovolemia did constrict the peripheral vasculature (4%), but did not constrict the cerebral vasculature. These studies of regional vasomotor responses are consistent with earlier studies of systemic responses in resting human subjects.

However, since sympathetic ganglionic stimulation at 8-15 pulses/sec can increase peripheral resistance 12-fold, it is unlikely that the observed vasomotor response to hormonal NE contributes significantly to circulatory homeostasis.

264.7 LOCUS COERULEUS NEURONS: SPONTANEOUS ACTIVITY IN BRAIN SLICES FROM NAIVE AND MORPHINE-DEPENDENT RATS. R. Andrade, C.P. VanderMaelen, and G.K. Aghajanian, Dept. Psychiat. and Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508.

In vivo studies in the rat have shown that noradrenergic locus coeruleus (LC) neurons are spontaneously active and are inhibited by morphine (MS). Repeated administration of MS results in tolerance as reflected by the eventual return over time of spontaneous activity (Aghajanian, Nature, 276:186-188, 1978). Recently, locus coeruleus neurons studied in guinea pig brain slices were found , by in large, not to be spontaneously active (Pepper et al., Exp. Brain Res., 45: 29-37, 1982). The aim of the present study was to examine whether LC neurons have spontaneous activity in rat brain slices and to use this preparation as a tool to study tolerance to opiates in the LC. This latter purpose is particularly well served by the brain slice preparation as it allows for a fast washout of tissue morphine in slices derived from tolerant animals and permits precise quantification of dose-response curves in a deafferented preparation.

Albino rats (100-250 g) were used in these experiments. Coronal slices (400 μm) were cut on a Sorvall tissue sectioner at the level of the LC. Brain slices were rapidly transfered to a humidified chamber continuously aerated with 95% O_2 and 5% CO_2, and placed on a Kimwipe strip saturated with flowing artifical CSF (NaCl, 130 mM; KCl, 5 mM; NaH_2PO_4, 1.25 mM; $NaHCO_3$, 24 mM; $CaCl_2$, 2.0 mM; $MgSO_4$, 1.5 mM; D-Glucose, 10 mM). Single unit recordings were made from LC neurons in slices from naive and dependent rats (one 75 mg MS subcutaneous pellet/day for 4 days).

LC neurons were spontaneously active in the rat brain slice under our conditions. They exhibited a characteristic long-duration positive/negative/positive waveform similar to that seen in vivo and regular firing rates ranging from 0.2 to 5 spikes/sec. Intracellular recordings, as in vivo, showed a marked post-activation inhibition accompanied by a prolonged after-hyperpolarization. LC neurons from both naive and dependent animals were inhibited by MS in a dose-dependent and naloxone reversible manner. However, substantially higher concentrations of MS were required to inhibit LC neurons from dependent animals. Firing rates in naive and dependent LC neurons were found to overlap. These findings suggest that rat LC neurons possess intrinsic pacemaker potentials (automaticity) and that tolerance to MS in the LC is at least partially mediated through a densensitization of LC opiate receptors. (Supported by Grants MH-17871, MH-14276, GM-07324, and the State of Connecticut).

264.8 DUAL ACTION OF NOREPINEPHRINE IN HIPPOCAMPUS. D. V. Madison† and R. A. Nicoll. Depts. of Pharmacology and Physiology, and Graduate Program in Neuroscience†, University of California, San Francisco, CA 94143.

The hippocampal slice preparation and intracellular recording have been used to examine the action of Norepinephrine (NE). The action of NE is twofold: 1) NE reduces IPSPs, and 2) NE blocks accommodation of spike firing of hippocampal pyramidal cells. The NE blockade of IPSPs results in an apparent increase in the size of the orthodromic EPSP which can result in multiple discharge. The block appears to occur at the level of the interneuron, since the reversal potential and action of iontophoretically applied GABA are not affected by NE.

When pyramidal cells are depolarized either by iontophoretically applied glutamate, or by constant current pulses applied through the recording electrode, they fire a few spikes and then remain silent for the remainder of the stimulus. NE abolishes this accommodation so that cells fire continuously to depolarizing stimuli. This effect occurs even when NE hyperpolarizes the membrane and inhibits responses to threshold depolarizations. NE also blocks Ca^{++}-activated K^+ conductance ($G_{K(Ca)}$). These effects are mediated by beta adrenergic receptors. Bath application of Ca^{++} antagonists, or intracellular injection of the Ca^{++} chelator, EGTA, block the $G_{K(Ca)}$ and also accommodation. Therefore, the block in accommodation by NE appears to be due to the blockade of the $G_{K(Ca)}$. However, NE does not reduce calcium spikes in these cells, and therefore must have its antagonistic action on $G_{K(Ca)}$ at some step after Ca^{++} entry into the cell. In addition, $G_{K(Ca)}$ and accommodation are blocked by extracellular application of the cyclic AMP analogue, 8-bromoadenosine 3',5'-cyclic monophosphate, or by intracellular injection of cyclic AMP, with no reduction of the Ca^{++} spike. Agents which directly activate adenylate cyclase also mimic these actions of NE.

Both the disinhibitory action, and the blockade of accommodation by NE, would enhance the responsiveness of the pyramidal cell to excitatory input. These actions combined with the membrane hyperpolarization would serve to inhibit weak inputs, while enhancing strong excitatory inputs, thereby increasing the "signal-to-noise" ratio in these CNS neurons.

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264.9 INCREASED BLOOD PRESSURE RESULTS IN A CENTRAL NEUROGENIC DECREASE IN CEREBRAL CAPILLARY PERMEABILITY TO WATER. R.T. Ling* and B.K. Hartman. Department of Psychiatry and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

The central noradrenergic system has been hypothesized to mediate intracerebral autonomic function. One such function may be to regulate brain fluid homeostasis in response to alterations in mean arterial blood pressure (MABP). It was previously shown in the monkey (Raichle and Grubb, Fed. Proc. 37:242, 1978) that an increase in MABP results in a decrease in cerebral capillary permeability to water. This observation has been extended to the rat system and shown to be neurogenic via a pathway including the visceral afferent system and the central aminergic system, which is known to innervate the cerebral microvasculature.

The extraction fraction of water across the cerebral capillary (Ew) was evaluated using the dual label intravenous injection method using C14-butanol as the reference tracer and H3-water as the test tracer (Clark et al., J. CBF and Metab., in press 1982). Ew becomes an index of capillary permeability to water because cerebral blood flow is not altered by the pressure changes in these experiments.

MABP was increased pharmacologically by intravenous administration of metaraminol or angiotensin II to produce an average increase of 40mm Hg for approximately 2 min. Ew responded by decreasing ($P<.001$) from a control value of .641 ± .01 to .537 ± .03. The magnitude of the decrease in Ew was independent of the pressor agent used. This response was completely blocked by surgical deafferentation of the peripheral baroreceptors at the carotid sinus and at the aortic arch. The data indicates two findings: (1) The effect on Ew was not due to a pharmacological effect on the cerebral microvasculature, and (2) It required centrally directed neurogenic information. Furthermore, bilateral superior cervical ganglionectomy did not affect the response of Ew. Therefore, this phenomenon is not the result of a centrally mediated peripheral sympathetic reflex arc.

Finally, selective destruction of the central noradrenergic system with 6-hydroxydopamine pretreatment resulted in complete abolition of the decrease in cerebral capillary Ew in response to increase MABP. This observation indicates that the phenomenon is indeed mediated by the central noradrenergic system. The decrease in permeability would tend to prevent fluid movement into the brain under the condition of increased pressure. These results represent a direct demonstration of the central noradrenergic system mediating an autonomic function in response to a physiological stimulus.

264.10 REGULATION OF BRAIN (Na^+,K^+)-ATPase BY ALPHA-1 NORADRENERGIC RECEPTORS. A.C. Swann, Dept. of Psychiatry, University of Texas Medical School, Houston, TX 77025.

(Na^+,K^+)-ATPase (NKA) is stimulated by norepinephrine in vitro and by acute noradrenergic stimulation in vivo (Life Sci. 28, 251). We have previously shown that repeated treatment with piperoxane or yohimbine increase NKA and ouabain binding to NKA in rat cerebral cortex, but the specificity of this effect for norepinephrine or for noradrenergic receptors is not established. The present experiments examined effects of locus coeruleus (LC) lesions and noradrenergic receptor blockade on the stimulation of NKA by repeated yohimbine. LC lesions decreased NKA and ouabain binding on the lesioned side. Pretreatment with yohimbine (2 mg/kg ip twice daily for four days) increased NKA and ouabain binding significantly only on the nonlesioned side. Pretreatment with prazosin, a selective antagonist of alpha-1 noradrenergic receptors, prevented the stimulation by yohimbine. The beta-antagonist propranolol did not prevent stimulation by yohimbine. The NKA that is stimulated by repeated yohimbine thus appears to be at least largely located on postsynaptic cells, and stimulation requires binding of norepinephrine to alpha-1 receptors.

265.1 SENSORY EVOKED POTENTIALS IN THE RAT DENTATE GYRUS REFLECT PROCESSING OF DIFFERENT TYPES OF INFORMATION. M.O. West, E.P. Christian* and S.A. Deadwyler*. Dept. Physiol. and Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

In previous reports (Science 211: 1181, 1981) we have described a sensory evoked potential recorded from the dentate gyrus of the hippocampal formation in freely moving rats. We are now in a position to present a more complete classification of the variables involved in producing the amplitude fluctuations in each component of this potential. Chronically implanted adult male Sprague Dawley rats were trained to a criterion of 100% responding to the rewarded tone (CS+) and less than 30% to the non-rewarded tone (CS-) in a differential discrimination paradigm. Tones (2.4 or 3.5 kHz) were presented in a random series at intervals of 30 to 90 sec, each with equal likelihood of occurrence on any given trial. Averaged tone evoked potentials recorded from the outer molecular layer (OM AEP) were constructed by means of a sorting procedure based on preceding trial sequence (Neurosci Lett. 28: 319, 1982). The three major components of the OM AEP (N1, N2 and P2) were related to specific aspects of the conditioning paradigm. The amplitude of the N2 component was directly related to the conditioned relevance of the tones, while fluctuations of P2 amplitude appeared to reliably reflect the occurrence of the CS+ vs the CS- on any given trial. One of the more interesting features revealed by extensive sequential analysis was the dynamic fluctuation in the amplitude of the perforant path mediated N1 component as a function of whether the preceding trial series consisted of CS+ vs CS- trials. We have determined that the critical variable in determining the trial-to-trial variation in N1 amplitude is the precise order of CS+ and CS- trials in the immediately preceding three trial sequence. The largest changes in N1 amplitude were produced when the preceding three trial sequence showed shifts toward or away from runs of all Cs+ or all CS- trials. Negligible fluctuations were observed during static conditions: N1 remained at maximum amplitude when the preceding three trials were all CS-, at minimum amplitude when all three were CS+, and at an intermediate amplitude when preceded by alternating CS+ and CS- trials. These results therefore suggest that neuronal transmission in the hippocampus and dentate gyrus may be regulated by endogenous brain mechanisms that are not related to specific parameters of the external sensory stimulus, but rather to cognitive processes which may reflect more abstract features of the conditioning situation. (Supported by NS 18288).

265.2 PROGRESSIVE ABNORMALITIES OF THE AUDITORY EVOKED POTENTIALS (AEP) AND THEIR RELATIONSHIP TO AUDIOGENIC SEIZURE(AS) PROPENSITY IN wl-WABBLER LETHAL MUTANT MUS MUSCULUS(C57Bl/6J). T.E.Sowa*, R.L. Curtis, M.J. Hosko, Jr., and F.D. Anderson. Department of Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226

The wl-Wabbler Lethal mutant(Dickie, et al.,J.Hered.43:283-286, 1952) on the inbred strain C57Bl/6J displays progressive motion tremor, broad base, tonic flexor spasms of the hind limbs on suspension by the tail and susceptibility to grand mal seizures from 13-14 days post partum (pp). MD 50 is 32 days on this genome.

AS"priming" under the conditions of Hall(1947) and Henry (Sci 158:928-940,1967) was confirmed for controls exposed at 19,24,29 and 34 days pp. wl homozygotes entered and survived the run and clonic stages of AS at these ages. The tonic stage was rarely observed.

Myelin (Anderson and Harman, Neurol.11:676-680,1961) and axonal degeneration (Carroll and Curtis, Anat.Rec. 199:284A,1982) has been demonstrated in many systems of wl/wl including vestibular pathways. Via silver techniques progressive axon,pre- and terminal degeneration was observed in the cochlear nerve, ventral cochlear nucleus, superior olivary complex and reticular formation (RF) of the medulla and pons. Mild degeneration was present in the lateral lemniscus(LL), deeper regions of the dorsal cochlear nucleus(DCN) and central nucleus of the inferior colliculus(IC),

The AEP of homozygous wabbler animals at the above ages differed significantly from controls in latency and amplitude. P_{III} was delayed and of low amplitude or masked by P_{IV} at later ages. The amplitude of the P_{IV-V} complex was significantly greater than controls at earlier ages and diminished as did all potentials with increasing age. Similar results were obtained with pentobarbital and chloral hydrate. The loci of origin of AEP peaks I-V were confirmed in mutants and controls by electrode studies with histological verification.

At 34 days homozygous wl animals demonstrated AS, severe diminution of AEP amplitudes and significant increase in P_{II} latency. Neuroanatomically, the DCN, LL and IC remained relatively intact. Direct projection of the DCN to IC have been described in the cat (Osen, JCN 144:355-372,1972). Willott and Lu (Exp Neurol 70:288-299,1980) concluded that connections between IC and RF of the mesencephalon were necessary for AS to occur. It is possible that continued AS propensity by the mutant in spite of massive degeneration in portions of the auditory circuitry, may result from receipt of auditory coded signals by the IC via DCN.

Supported by NIH NS-07680,N01-RR-8-2134, The Evan and Marion Helfaer Foundation, Shriners Hospital for Crippled Children, and the MCW Department of Neurology, Neurosurgery, Physical Medicine and Rehabilitation.

265.3 THREE-DIMENSIONAL AUDITORY BRAIN STEM RESPONSES RECORDED FROM THE CAT. W.H. Martin*, J.N. Gardi, M.G. Randolph* and Y. Sinninger*. Coleman Laboratory, Univ. of Calif., San Francisco, CA. 94143.

Auditory brain stem responses (ABRs) have been recorded from the scalp of animals and humans. These potentials, when recorded using three orthogonally placed electrode pairs, result in a Lissajous figure representing three-dimensional (3-D) voltage activity of the ABR (Gardi, J.N., Martin, W.H. and Jewett, D.L., J. Acoust. Soc. Am., 68:s19, 1980; Williston, J.S., Jewett, D.L. and Martin, W.H., Brain Res., 223:181, 1981).

Three-channel ABRs were recorded from anesthetized (sodium pentobarbitol) cats in response to clicks and/or tone bursts. Auditory stimuli were presented monaurally to each ear and binaually at intensity levels from 40 to 80 dB impulse SPL. Electrodes were placed at mouth-nuchal ridge (X), left and right mastorids (Y) and vertex and throat (Z), with variations for specific mapping studies. Single-channel responses were combined using a specialized computer graphics program. Data points were displayed on an oscilloscope in three dimensions for analysis of waveform, planarity of data points and position of data points relative to the X,Y,Z axes.

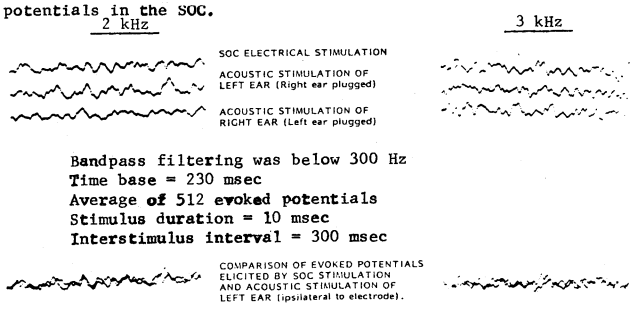
Among the results were the following: 1. At 70 dB impulse SPL, the 3-D ABR recorded from the cat may be divided into as many as thirteen sequential planar-curves. 2. The order of these planar-curves, as well as their relative spatial locations, was consistent within and across animals. 3. As stimulus intensity was decreased, complexity of three-dimensionally recorded waveforms also decreased; resulting in fewer planar-curves, each of which exhibited an increased latency and reduced size. 4. Contrary to earlier findings, position of planar-curves in space also changed as a function of changes in stimulus intensity. 5. Formation of planar-curves was not dependent on orthogonallity of recording electrode pairs. Although planar-curve position changes were observed, the number, order, shape and latency of non-orthogonally recorded planar-curves did not change. 6. Monaural stimulation to the left ear produced a mirror image of the entire 3-D ABR recorded to stimulation to the right ear. 7. Binaural stimulation produced a collapse of activity towards the midline, especially of information in the "Y" axis, resulting in significant position changes in space. 8. Presentation of tone burst stimuli resulted in planar-curves which differed from click-evoked planar-curves; demonstrating frequency dependency of 3-D ABRs evoked by tonal stimuli.

These results suggest: 1. Three-dimensionally recorded ABRs provide a more complete record of neural acitivity along auditory brain stem pathways than could be obtained from records obtained from any one recording configuration. 2. Multiplicity of reliablly recorded planar-curves suggests an underlying complexity of neural generators that may not be fully appreciated in single channel records. 3. Manipulation of stimulus variables, especially intensity and frequency, result in predictable changes in planar-curve shape, position and size, suggesting that underlying neural organization patterns (including tonotopicity and stimulus strength) may be coded by planar-curve formation patterns.

265.4 FREQUENCY DEPENDENCE OF CORTICAL EVOKED POTENTIALS ELICITED BY ACOUSTIC AND ELECTRICAL STIMULATION OF THE SUPERIOR OLIVARY COMPLEX. Dale Russell Berard, Department of Psychology, University of California, Davis CA 95616.

Cortical evoked potentials elicited by acoustic and electrical stimulation of the superior olivary complex (SOC) were compared for preservation of waveform similarity. Vertex scalp recordings from rats, which had previously discriminated electrical stimulation of the SOC, showed frequency dependent similarity between 0.2 to 3.9 kHz. SOC stimulation current was maintained at 20 peak μA, a level which provided good frequency judgement during behavioral assessment. As shown below, a 3 msec time lag was found between 70 dB acoustic and SOC stimulation produced waveforms which can be accounted for by forward stimulation of the auditory pathway. 3 msec post-stimulus events correspond to SOC activity in rat brain stem evoked potentials. Recordings from control rats with electrodes directed 1.2 mm medial, lateral and dorsal to the SOC did not show any waveform similarity with records elicited by acoustic stimuli.

Electrical stimulation of the SOC was patterned to simulate the frequency following response (FFR), an integrated extracellular field potential generated by the volleys of phase locked input from the anterior ventral cochlear nucleus. The conjunction of synchronous FFR fields with the spacial tonotopic organization of the mid-SOC may select patches of SOC cell membrane which index and correlate low frequency representations in the auditory brain stem. Because bilateral cochlear damage does not prevent behavioral discrimination of SOC electrical stimulation, feedback to the ear is not necessary for perceptual significance of simulated FFR extracellular field potentials in the SOC.



265.5 RATE-LIMITING TEMPERATURE EFFECTS ON AUDITORY EVOKED RESPONSES. G.T. Rossi and R.H. Britt. Neurosurg. Div., Stanford Med. Sch., Stanford CA 94305 and Palo Alto Veterans Hospital, Palo Alto CA 94304.

Pentobarbital anesthetized cats were placed on total cardiopulmonary bypass as described by Britt and Rossi (Neuroscience Abstracts, 7:54, 1981). After initiating bypass, extracorporeal blood was cooled down to 20°C to produce systemic hypothermia. Brainstem auditory evoked responses (BAERs) to monaural clicks (90 dB, 10/sec) were filtered (150-3KHz) and differentially averaged between vertex and mastoid electrodes. Temperature of the left parietal lobe was measured using a thermocouple probe while serial BAERs were recorded at 2 minute intervals as brain temperature was lowered to 20°C. This correlation between BAERs and brain temperature was sufficiently accurate to begin to describe a mathematical temperature-latency relation.

Two significant temperature effects were produced. First, latencies increased as brain temperature was lowered. Between 37° and 32°C only small changes of less than ±0.04 msec were recorded. Below 32° plots of latency vs temperature for each wave were found to be exponential functions. Second, wave peak amplitudes initially increased; then decreased as temperature was lowered. Amplitudes increased to their maximum values near 32°C, then decreased and were lost completely below 23°C. Amplitude-temperature plots for all 5 waves were parabolic in shape with different slopes (μ V/°C). Arrhenius plots were used to determine whether the auditory system could be described in a temperature dependent manner. The inverse log latency (or log rate) was plotted against reciprocal absolute temperature as a first step. This produced a series of lines with equal slopes. This slope produced a single exponential value which completely matched the log rate (and therefore, latency) for all 5 waves during systemic (core) hypothermia. This exponent was equal to 5.0 ±0.5 kcal/mole °C for cooling rates and temperatures used in these experiments. Rewarming restored the BAERs to the original latencies and amplitudes. Similar results were obtained for sequential cooling and rewarming cycles.

This study suggests the BAER is significantly rate-limited by a temperature-dependent event. This event may occur either in the receptor organ or elsewhere in the ascending auditory pathway, examples of which include membrane permeability or synaptic delays. All BAER waves followed a single exponential latency-temperature function indicating that the temperature dependence is not restricted to a single neural generator.

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265.6 CURRENT SOURCE DENSITY ANALYSIS OF THE SOMATOSENSORY EVOKED POTENTIAL IN THE CAT. P.B. HOELTZELL* and R.W. DYKES

The sequence of potential changes following a brief punctate tactile stimulus was studied in somatosensory cortex of the cat. Each animal was anesthetized with nembutal and a standard craniotomy was performed. Fixed amplitude 0.1s duration mechanical stimuli were delivered to the forearm at 2s intervals. These stimuli produced restricted loci of activity in primary somatosensory cortex in which numerous action potentials were detected with low impedance tungsten electrodes at depths from 500-1200μm below the pia. Once these loci were identified a 1-3mΩ glass electrode was substituted for the metal one, and rows of penetrations separated by 200μm were made in rostrocaudal or mediolateral directions. At each penetration site potentials were recorded at 100μm steps from 100μm to 1500μm below the pia. This procedure produced a three-dimensional matrix of evoked potentials. Electrolytic lesions were left at selected points so that serial reconstructions could be correlated with the data from a volume of cortex centered on the focus of activity.

A Z-80 microprocessor system controlled mechanical stimulation and positioning of the electrode and collected, digitized, and stored the data on disk for further analysis. When the cat was sacrificed, the tip of the recording pipette was left in the focus and the portion of cortex under study removed. Serial sections were cut parallel to the rows of penetrations. From these, camera lucida drawings were generated showing the cortical layers and cytoarchitectonic fields. Using the electrode lesions as references, field potentials and three-dimensional current source density data were superimposed on the reconstructions.

Analysis of the evoked potentials revealed three types of depth profiles: type 1; a classical spatial dipole with the surface positive and initially deep negative waveforms, type 2; initially positive waveforms both superficially and deep, and type 3; initially negative waveforms throughout, with a maximum deep in the cortex. Multiple loci were found, each corresponding to a particular cytoarchitectonic field. Current source density analysis revealed that all of these evoked potential patterns could be accounted for by the activity of two distinct, spatially-separated, populations of cells. One displayed a strong-superficial source and deeper sink dipole only in layer III, corresponding to laminar field potential type 1 or type 2, and the other population displayed a variable weak source-sink pair corresponding to the maximum intensity of the type 3 potential and the type 1 negative peaks in layers IV and V.

265.7 LINEAR AND NONLINEAR ANALYSIS OF VISUAL EVOKED POTENTIALS OF THE HOODED RAT. C. Harnois and I. Bodis-Wollner. Department of Neurology, Mt. Sinai School of Medicine, New York, NY 10029.

It is well known that linear and nonlinear properties are useful for classifying retinal and lateral geniculate nucleus cells of cats (1,2) and monkeys (3). We studied pattern visual evoked potentials (VEP) of the hooded rat, using a nonlinear system analysis developed by Victor and Knight (4). VEPs were recorded with chronically implanted electrodes placed over area 17 and the postbregmatic region. The stimulus was a sinusoidal grating of 0.1 cpd and 50% contrast displayed on a CRT screen which subtended 46° of visual angle at the eye. The grating was reversed with a sum of temporal sinusoids. A linear system yields responses only at the input frequencies (f1), while a quadratic nonlinearity would also produce response components at frequencies f1 ± f2 where f1 and f2 are frequencies in the input sinusoidal sum. The frequencies were chosen so that the input frequencies and sums and differences of the input frequencies were distinct. Results show that the hooded rat VEP contains significant nonlinear responses. First order (linear) responses to temporal input frequencies were smaller than the second order (nonlinear) responses. Responses to difference frequencies were much higher than to sum frequencies.

We have already reported that alphamethylparatyrosine (AMT), a dopamine (DA) depletor, and haloperidol, a DA blocker, cause a delay of the VEP at both low and high temporal frequencies and that these changes can be reverted by apomorphine, a DA agonist (5). This study suggested that the VEP latency changes in the rat, and perhaps those observed in Parkinson's disease, may be caused by dopaminergic dysfunction. The site of this dopaminergic deficiency is not known. It could be at the retinal level, involving amacrine cells, or in cerebral catecholamine structures, or both. Amacrine cells are thought to be the important nonlinear element in the proximal retina (6). The reversible effect of DA deficiency on the nonlinear characteristics of the VEP will be presented.

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265.8 THE EFFECT OF CONTRAST ON THE VISUAL EVOKED POTENTIAL OF THE HOODED RAT. I. Bodis-Wollner and C. Harnois. Departments of Neurology & Ophthalmology, Mt. Sinai School of Medicine, New York, N.Y. 10029.

For studying the rat visual evoked potential (VEP), few have employed patterned stimuli, widely used in humans, monkeys, and cats. Using patterns we have studied the effects of refraction, spatial and temporal frequencies on the rat VEP (Onofrj et al. Physiol. Behav. 28: 227-230, 1982). Now, we report on the contrast response of the rat. Seventeen male adult hooded rats had VEPs recorded with chronically implanted electrodes placed over area 17 and the postbregmatic region. The stimulus was a vertical (and in some experiments a horizontal) sinusoidal grating pattern of 0.1 cpd, counterphase modulated at the rate of 1 Hz (transient) or 8.4 Hz (steady-state). Contrast was varied in small steps ranging from 3.4 to 90%. In the transient pattern VEP of the rat, we could observe the typical triphasic (NPN) VEP well known from humans. With changing contrast from 10 to 55%, the latency of the major positive wave decreases linearly from 100 to 80 msec, and its amplitude increases from 2.5 to 12 μV. From then on, latency and amplitude remain constant. For the steady-state VEP, both amplitude and phase of the harmonic components were evaluated using Fourier analysis. Using counterphase modulation, the response was dominated by the second harmonic. The amplitude of the second harmonic as a function of the contrast does not show a monotonic linear function; rather it reveals a shallow slope at low, and a steep slope at higher contrasts. The phase plot shows a phase shift of 296° for a change of contrast from 3.4% to 80%. By extrapolating to "threshold" and using different spatial frequencies, we can chart the contrast sensitivity function of the rat. The VEP characteristics of the hooded rat are similar to those of the human and monkey at low spatial frequencies.

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265.9 THE PATTERN ERG IN HUMANS: DEPENDENCE ON PATTERN CONTRAST, SPATIAL FREQUENCY, AND TEMPORAL FREQUENCY. Curtis L. Baker, Jr. and Robert F. Hess*, Physiological Laboratory, Cambridge University, Cambridge, U.K.

Using gold foil electrodes (Arden et al, 1979), we have recorded the human electroretinogram in response to a sinusoidal grating which is sinusoidally counterphase-flickering. With signal averaging time-locked to the temporal flicker cycle, a narrow-band signal at the second harmonic of the temporal frequency is recorded, typically 0.1 - 5.0 μV amplitude.

The temporal frequency response shows a bandpass behaviour peaking close to 8 Hz, which was chosen as a standard temporal frequency for the remaining work.

Spatial frequency response was also bandpass, centered at ca 5.0 cycles per degree. The low spatial frequency roll-off was observed for all combinations of sinusoidal vs. square-wave temporal and spatial waveforms. This spatial bandpass characteristic shifted systematically with retinal eccentricity, such that a pattern viewed 34° eccentric gave a spatial frequency bandpass centered at ca 0.5 cycles per degree. The latter result cannot be explained by eccentric ametropia: tests with a series of trial lenses revealed no optical correction which could abolish the high spatial frequency roll-off.

The pattern ERG amplitude shows a monotonic, nearly linear increase with contrast, regardless of temporal frequency, spatial frequency, spatial or temporal waveform, or retinal eccentricity.

These results, particularly the spatial frequency bandpass characteristic and its dependence on eccentricity, are consistent with the idea that the pattern ERG reflects activity of post-receptoral processes (e.g., retinal ganglion cells), as proposed by Arden et al (1980) and Maffei and Fiorentini (1981).

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265.10 BRAIN ELECTRICAL ACTIVITY MAPPING (BEAM) OF SCHIZOPHRENIA. J. Morihisa, F. Duffy and R. J. Wyatt* (SPON: A.P. Oliver)*. Adult Psychiatry Branch, NIMH, St. Eliz. Hosp., Wash. D.C. 20032 and Dept. Neurology, Harvard Medical School, Boston, MA.

Although there are many reports of electroencephalography (EEG) and evoked potential (EP) abnormalities in schizophrenic populations, there is no agreement as to which of these abnormalities predominate or are important. The use of schizophrenic patients who are taking medication and the examination of only certain regions or electrodes may have contributed to this problem. However, multiple lead EEG and EP investigations which look at all regions of the scalp generate an enormous amount of data. The BEAM technique uses topographic representation to condense and summarize this information in an easily assimilated and intuitively meaningful manner. The BEAM acquires data from all 20 standard 10-20 electrodes simultaneously which is processed off line on a PDP 11-60. Evoked potential and spectral analysis data is presented as a color map within a graphic outline of the head. Spectral energy in each of the classic EEG bands and of EP voltage at any 4 msec epoch after stimulus onset may be displayed as a color map. Each color represents a voltage range. The ability of the computer to create a new video image every 100 msec allows the display of brain electrical activity in space as topographic maps as well as in time in the form of cartooned images. A movie of evoked potentials will be demonstrated.

Data will be presented from our study of 11 normal subjects, 11 drug-free schizophrenic patients and 14 medicated schizophrenic patients. The subjects are matched for age, sex and handedness. The schizophrenic patients fullfilled Research Diagnostic Criteria for this diagnosis. Unmedicated patients were drug free for at least 4 weeks and medicated patients were on drugs for at least 4 weeks.

A statistical treatment significance probability mapping (SPM) will be used to demonstrate areas of greatest regional difference. The drug-free schizophrenic patients in general demonstrated increased delta (0.0-3.7 Hz), greatest frontally when compared to the normal controls. Drug free schizophrenics demonstrated increased beta (28.0-32.0 Hz) whose SPM of regional difference was greatest post frontally. Medicated schizophrenics also demonstrated regional differences in the same delta and beta bands.

The audio evoked response (AEP) of drug free schizophrenics as well the medicated schizophrenics showed right sided area of regional topographic difference at 100 msec following stimulus onset when compared to the same topographic region in normal controls as delineated by SPM. The visual evoked response (VEP) of medicated schizophrenics shows an area of frontal regional difference compared to normals at 400 msec following stimulus onset as delineated by SPM.

The implications of these electrophysiological findings to the investigation of schizophrenia will be discussed with particular attention to cerebral blood flow studies. In addition, the compatibility of this research approach with other topographic and tomographic (CT, PET) research modalities will be examined.

265.11 STUDIES ON NORMAL PARAMETERS OF PERIPHERAL NERVE CONDUCTION WITH REFERENCE TO SEX AND AGE IN HUMANS. N.M.Khalil*, Ajib-Ali* and M.I.Hilmy. Dept. of Physiology, Mosul Univ. Med.Coll. Mosul, Iraq.

Measurement of peripheral nerve conduction parameters could vary in different electromyographic (EMG) machines used, methodology applied and may be there are regional variation. The objective was justified to establish normal values to be used in hospitals in this country. The commonly used nerves for routine diagnostic tests are the median ulnar, peroneal and sural nerves were studied in 233 subjects symptoms-free of neuromuscular diseases aged 12-76 years. Bilateral sensory and motor conduction measurements of the median and ulnar nerves were made on 124 subjects of both sexes. A similar study was made on the remaining subjects where the peroneal and sural nerves were used. The DISA-1500 Digital EMG machine was used. The results indicated that no significant differences were noted in nerve conduction velocity (NCV), distal motor latency (DML), sensory latency (SL), and amplitude of nerve action potenial (NAP) between right and left sides of either sex and also between males and females for all nerves tested. There was no significant difference in motor CV of the median and ulnar nerves while that of the median was 7 m/sec greater than that of the peroneal nerve ($P < 0.001$). With age, the NCV slowed down, the amplitude of NAP reduced and the DML and SL were prolonged. In general, the data obtained are in agreement with the literature.

266.1 GROWTH-FACTOR-INDUCED PHOSPHOLIPID METHYLATION IN NERVE GROWTH CONES. K.H. Pfenninger, M.P. Johnson* and L. Ellis, Dept. of Anatomy & Cell Biology, Columbia Univ. P&S, New York, NY 10032.

In order to study whether nerve growth factor (NGF) stimulates phospholipid methylation in sprouting PNS neurons, cultures of rat superior cervical ganglia were first deprived of NGF and loaded with [^{3}H-methyl]-methionine for a total of 90 and 45 min, respectively. Cultures were then pulsed with medium containing 2.5S NGF, and the reaction was stopped at various short intervals thereafter by the addition of ice-cold 10% trichloroacetic acid. Distal neurites and growth cones (GC fraction) were then microsurgically separated from proximal neurites and perikarya (P fraction), and the two fractions were extracted with chloroform-methanol. Aliquots of the extracts were used (i) for the determination of total phospholipid, (ii) for the measurement of radioactivity and (iii) for thin-layer chromatography (TLC). While the P fraction showed no NGF-induced changes in the specific radioactivity of phospholipid, such changes were evident in the GC fraction: Within seconds the specific radioactivity of phospholipid was increased several fold over baseline levels, reaching a peak 10-15 sec after onset of the stimulus and falling back to control levels in about 60 sec. TLC revealed NGF-induced stepwise methylation of phosphatidylethanolamine to phosphatidylcholine. Furthermore, a small peak of radioactive lysophosphatidylcholine was detected, indicating the presence of phospholipase A_2 (PLA2) near the methylation site. The activation of PLA2 via transmethylation and Ca^{2+} influx is believed to trigger histamine release by exocytosis in mast cells (e.g., Hirata & Axelrod, Science 209:1082, 1980). Thus, we propose that NGF stimulates exocytotic membrane addition in PNS growth cones by a similar enzyme cascade. This process of localized membrane expansion may be a part of the mechanism of neurite chemotaxis. Further analyses of the transmethylation-PLA2-activation cascade in growth cones are in progress.

The demonstration of NGF-stimulated phospholipid transmethylation in peripheral neurons raises the possibility that the response to putative growth factors of other neurons, e.g., those of CNS origin, may be mediated by similar intracellular mechanisms. To investigate this possibility, we have developed chemically defined media that promote, in cerebral cortical explants, vigorous outgrowth of neurites comparable to that obtained with NGF in PNS neurons. The transmethylation response of CNS growth cones to presumptive growth factors is currently being investigated. (Supported by NSF grant BNS 14071 and a NRSA to L.E.)

266.2 ISOLATION OF NERVE GROWTH CONE PARTICLES FROM RAT BRAIN. L. Ellis, L.B. Friedman*, M.P. Johnson*, S. Somlo* and K.H. Pfenninger. Department of Anatomy and Cell Biology, Columbia Univ., P&S, New York, NY 10032.

To facilitate the biochemical analysis of the axon's growing tip, we have developed a method for the preparation of a subcellular fraction highly enriched in pinched-off fragments from nerve growth cones. The brains of 17-day-gestation rat fetus are homogenized, and a postnuclear supernatant is prepared. This is layered on a discontinuous sucrose density gradient and spun to equilibrium. A band of very low density contains almost exclusively membrane-bound particles of approximately 0.3 μm diameter. These contain organelles typical of nerve growth cones: microfilaments, large clear vesicles, 120nm dense-core vesicles, agranular reticulum, but no ribosomes. By freeze-fracture, these elements, henceforth referred to as "growth cone particles" (GCPs), have a plasmalemma characteristically poor in intramembranous particles (cf. Pfenninger and Bunge, 1974).

The direct identification of GCPs as elements of growth cone origin is accomplished as follows: Primary explant cultures are grown until a dense halo of neuritic growth extends well beyond the explant. These cultures are labeled with ^{35}S-methionine, the distal neurites with growth cones are microdissected from the cultures, and this material is homogenized and processed with the fetal brains as above. By electron microscopic autoradiography of the GCP fraction, occasional GCPs are found to be heavily labeled with silver grains, thereby demonstrating the co-purification, with this subcellular fraction, of nerve growth cones grown in vitro .

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of the GCP fraction reveals approximately 40-50 major bands. By two-dimensional SDS-PAGE, the major proteins are identified as actin and β-tubulin. Other proteins thus far identified on the basis of electrophoretic mobility include fodrin and calmodulin. GCPs can be lysed in 6mM Tris buffer (pH 8.1) and a membrane fraction prepared from them. While the majority of proteins appear in the lysate, the washed membrane preparation has been found to contain a high proportion of lipid and a very simple protein pattern. By SDS-PAGE, one can detect only one major broad band (approx. 55kd) and less than ten minor bands. These proteins are not removed from the membranes with high salt and, therefore, are believed to be integral to the membrane. The further characterization of GCP membrane components, e.g., glycoconjugates, is currently in progress. (Supported by NSF grant BNS 14071 and by a NRSA to L.E.)

266.3 RETINAL GANGLION-CELL AXONAL OUTGROWTH UPON SUBSTRATA DERIVED FROM BASEMENT MEMBRANE SECRETING TUMOR AND CNS TISSUES. N.R. Smalheiser, S.M. Crain, and L.M. Reid*. Depts. Neuroscience and Molec. Pharm., Albert Einstein Coll. Med., Bronx, NY 10461.

Retinal and dorsal-root ganglion cell axons selectively ramified within their appropriate CNS target explants in vitro (tectum vs. spinal cord; N.R.S. et al, Brain Res. 208,'81; Devel. Brain Res. 2, '82). As these ramifications resembled neuritic patterns seen by other workers upon highly adhesive artificial substrata, we have suggested that CNS targets contain endogenous adhesive molecules distributed as a 'substratum' fostering appropriate ramifications. Alternatively, neurites might be ramifying in response to synaptic sites on target cells. We have studied this question further: A) by co-culturing retina with a presumed non-synaptic target rich in extracellular matrix; and B) by plating nonviable extracts of CNS tissues to form substrata.

A) Ganglion-cell axons emerging from 13-14 day fetal mouse retinal explants projected into living explants and small cellular clumps of EHS basement membrane secreting tumor (Orkin et al, J.Exp.Med. 145,'77) where they ramified extensively and adhered closely to basement membranes (BMs). Crude tumor extracts(crushed while freezing/thawing on dry ice x4) and EHS 'biomatrix' (tumor mince exhaustively extracted with saline, nucleases, and deoxycholate or Triton), when spread thinly and air-dried on glass coverslips or microwells, allowed explant attachment and excellent neurite outgrowth. Trypsin destroyed this activity. A glycoprotein purified from EHS BMs, laminin (gift of G. Martin, NIH), also fostered explant attachment and axonal outgrowth when air-dried at 10-1,000 μg/ml. Axons grew sharply confined to laminin dried as patterned streaks on collagen-coated coverslips. Anti-laminin antiserum inhibited attachment and outgrowth upon laminin substrata, dependent upon both laminin and antiserum concentration; anti-fibronectin had no effect. Anti-laminin (but not anti-IV collagen) disrupted 'biomatrix' BMs, whereas crude EHS extract BMs were unaltered. In initial tests, antiserum inhibited attachment to disrupted BMs. B)18 day fetal tectum and cord, stripped of meninges and prepared as crude extracts, both supported excellent retinal axon outgrowth. Axons grew to a lesser extent upon air-dried Triton X-100 supernatants of crude tectal extracts (spinal cord has not been fully tested), but not extract buffer.

These findings suggest that for at least one model target,the EHS tumor, retinal axon ramifications reflected adhesive interactions with specific molecules arrayed as a 'substratum' within the target tissue. Further work is needed to determine if this is also true for CNS target tissues. [This work was supported in part by NIH grants: CA-30117 to LMR and NS-14990 to SMC. We thank workers at the Laboratory for Developmental Biology and Anomalies, NIDR, NIH, for their help and for supplying reagents.

266.4 DEVELOPMENT OF CIRCUMFERENTIAL AXONS IN CHICK EMBRYOS: THE FIRST SPINAL CORD AXONS. J.A. Holley and R.J. Lasek (SPON: I.R.K. Abramof) Dept. Anatomy, Case Western Reserve Univ., Cleveland, OH 44106.

During development, axons grow along stereotypic pathways to reach often distant synaptic targets. At present, few studies have described in detail the early development of axonal pathways in vertebrates. Therefore, the early growth of spinal cord axons was studied by high resolution microscopic methods. A major set of early forming spinal cord axons was recently shown to grow in the transverse plane and along the lateral ventricular zone margin, and was termed the circumferential axonal pathway (Holley J.A., J. Comp. Neurol., 205:371, 1982). In order to study the growth of the earliest circumferential axons, the brachial spinal cord of a stage 17$^+$ chick embryo was serially thin-sectioned after conventional TEM processing. A total of 100 peri-sagittal sections were collected beginning at the lateral spinal cord margin. Montages of every fifth section were made, and outlines of the cell bodies and major cell processes were drawn on plastic overlays. Analysis of these cellular profiles after reconstruction showed that all long cellular processes were oriented in a precise dorsoventral direction. They were arranged in a parallel pattern of single or paired processes that coursed perpendicularly through the marginal zone, and appeared ultrastructurally to be immature axons. Seven of a total 24 circumferential axons had a terminal end at least partially within the series; each formed a well defined growth cone that projected ventrally. No obvious specialized structures such as channels, extracellular matrix or other (glial) processes were found ahead of these presumably growing circumferential axons. These findings showed that circumferential axons were the first axons to grow in, at least, certain regions of the chick spinal cord. Moreover, they grow in a highly ordered pattern from the outset that is oriented precisely orthogonal to both the radial processes and the later growing longitudinal spinal cord axons. Although no morphological basis of guidance was evident, further analysis at higher magnifications of the relationship between the growth cones and the adjacent end-feet of the external limiting membrane and surrounding neuroepithelial cell processes may yet offer insight into the guidance cues directing the pioneering circumferential axons.

266.5 DIFFERENTIATION OF SENSORY NEURONS IN THE PUPAL WING OF DROSOPHILA Marjorie A. Murray, Margrit Schubiger*, and John Palka. Dept. of Zoology, University of Washington, Seattle, WA 98195

The wings of Drosophila melanogaster are favorable material for asking how specific axon pathways are formed. Like other insect appendages, Drosophila wings carry an array of sense organs which detect chemical and mechanical stimuli. In the wild type wing, sensilla occur only on longitudinal veins 1 and 3 and their axons travel only in these veins to the CNS. The differentiation of the neurons occurs during the transition from larva to adult fly, a process taking 120 hrs. Using an antibody to HRP which binds to insect neurons, we have followed the differentiation of the sensilla on vein 3 (L3) in the developing pupal wing.

By 18 hrs after pupariation, the full adult complement of seven neurons is visible along L3, five with dendrites and two without. The five cells are obviously sensory neurons, but the function of the other two is at present obscure. They are evidently the cell bodies of two "extra axons" we previously encountered in EM cross-sections of pupal and adult wings and which we suggested might pioneer the L3 axon pathway.

As early as 6 hrs after pupariation, two neurons along the incipient L3 are stained with anti-HRP. Each has a dendrite reaching to the surface of the epithelium, and in some cases the cells have produced sprays of filopodia, evidence of axonogenesis. Faint staining is already seen at 2 hrs after pupariation.

It is possible to estimate the birthdates of sensilla-producing cells by treatment with heavy doses of X-rays. This causes blockage of mitoses following the irradiation, so that only cells which have undergone their final division are able to produce sensilla. This technique indicates that cells forming certain sensilla on the wing, including two on L3, are born even prior to pupariation, an observation consistent with the finding of two early-staining neurons in this vein. In general there is good agreement between the birthdates (birth-hours) estimated with the X-ray method and the slightly later time of differentiation seen with the anti-HRP staining.

Certain mutants of Drosophila add sensilla all over the wing and while axons from these supernumerary sensilla can travel in other veins, they appear to prefer L3, frequently crossing the intervein space to join it. What establishes the normal axon pathway in L3, and why are ectopic axons attracted to it? The first neurons to differentiate might establish the path; alternatively, the wing epithelium itself may contain pathway cues which any axon reaching it can read. We are currently attempting to distinguish between these possibilities.

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266.6 PATHFINDING BY NEURONAL GROWTH CONES IN GRASSHOPPER EMBRYOS: RELATIONSHIP OF GROWTH CONES AND THEIR FILOPODIA TO AXON BUNDLES. M.J. Bastiani, J.A. Raper, and C.S. Goodman, Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305

We are interested in understanding the mechanisms that guide neuronal growth cones during embryonic development. The final morphology of a neuron results in large part from a series of simple choices made by its growth cone during development. We have been studying the divergent choices made by the growth cones of the G and C neurons, sibling cells that arise from the second cell division of neuroblast 7-4 during grasshopper embryogenesis.

The growth cones of G and C both extend medially across the posterior commissure to a "choice point" at the lateral edge of the contralateral neuropil; up to this point the two cells are indistinguishable on the basis of morphology but can be distinguished by the position of their cell bodies. At this "choice point" the G and C growth cones make divergent choices: the G growth cone turns anteriorly, while the C growth cone turns posteriorly. Light and EM analysis has shown that at the "choice point", the G and C growth cones are within filopodial grasp of many axon bundles. However, the G and C growth cones choose to grow in a distinct lateral axonal pathway made of four other identified neurons: A1, A2, P1, and P2.

The G and/or C neurons were filled with HRP and serial EM reconstructions were made to characterize the relationship of the G and C growth cones and their filopodia to the axon bundle. (i) The filopodia from G and C appear to preferentially adhere to the axons of the lateral bundle before they make their divergent choices. (ii) Initially, the filopodia do not show any selectivity within the lateral bundle, or in the anterior or posterior directions. (iii) However, the growth cones do preferentially extend along particular axons within the lateral bundle. For example, the G growth cone extends anteriorly from the choice point on the posteriorly-extending axons of the P cells. The behavior of the filopodia suggests that (i) different axon bundles may be differentially labeled (supporting the "labeled pathways" hypothesis), and that (ii) there is a single label on the axons within an individual bundle. The eventual selectivity of the G growth cone within the bundle may reflect differences in the relative adhesivity of the newer (P cell) vs. older (A cell) membranes within the bundle. [Supported by the NIH, NSF, and the McKnight Foundation]

266.7 MUSCLE PIONEERS: LARGE MESODERMAL CELLS THAT ERECT A SCAFFOLD FOR DEVELOPING MUSCLES IN GRASSHOPPER EMBRYOS. E.E. Ball, R.K. Ho*, K. Kotrla*, and C.S. Goodman. Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305

In the development of vertebrate skeletal muscles, motoneuron growth cones often arrive in the periphery and enter the appropriately located masses of mesodermal cells before muscle fibers appear (e.g. Dennis, Ann. Rev. Neurosci., 1981). We find that the same sequence occurs in the grasshopper embryo. We are interested in what organizes the mesodermal cells into the appropriate muscles, and what guides the motoneuron growth cones to the appropriate mesodermal masses.

We have used monoclonal antibodies (mabs) to study the cellular events underlying muscle development in the body and limb buds of the grasshopper embryo, and have discovered a class of large mesodermal cells, called muscle pioneers, which appear early in development and erect a scaffold for later developing muscles. We discovered the muscle pioneers using the I-5 mab, an antibody which stains a subset of neurons and mesodermal cells in the embryo (Chang, Ho, and Goodman, Dev. Biol., in review).

(i) The muscle pioneers appear in the mesoderm early in development when the terrain is relatively simple and distances short. (ii) The muscle pioneers have different adhesive properties than other mesodermal cells in that they often flatten and adhere to the ectodermal epithelium. (iii) They extend large growth cones (much like neuronal growth cones) across the inner surface of the ectoderm (or its basement membrane) which stop and insert in the ectoderm at the appropriate places for the muscle insertion. (iv) By their prior insertion at the site of ectodermal invagination, they appear to be intimately involved in the morphogenesis of tendons. (v) Other mesodermal cells cluster around the muscle pioneers and later develop into muscle fibers, using the long processes and ectodermal insertions of the muscle pioneers as a scaffold for their development. (vi) The muscle pioneers appear to be an antigenically distinct cell type in the grasshopper embryo. The mes-2 mab specifically stains the muscle pioneers and no other cell type; for example, it does not stain the later developing muscle fibers that surround the muscle pioneers (this mab is from a joint Drosophila/grasshopper fusion by Kotrla, Jan, Jan, and Goodman, in preparation). (vii) Our results (see next abstract, Ho, Ball, and Goodman) suggest that motoneuron growth cones are guided to their appropriate targets by both axonal pathways and these muscle pioneers.
[Supported by the Australian Natl. Univ., McKnight Foundation, and NSF.]

266.8 GUIDANCE OF MOTONEURON GROWTH CONES BY AXONAL PATHWAYS AND MUSCLE PIONEERS IN GRASSHOPPER EMBRYOS. R.K. Ho*, E.E. Ball, and C.S. Goodman (SPON: J. Kuwada). Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305

Growth cones from motoneurons in the chick spinal cord take stereotyped pathways to their appropriate hindlimb muscles (e.g. Lance-Jones and Landmesser, Proc. R. Soc. Lond., 1981). Our observations in the limb buds of grasshopper embryos demonstrate a similar stereotyped pattern of axonal outgrowth by identified motoneurons. What cues guide motoneuron growth cones to their appropriate targets? We have visualized the motoneuron growth cones, their filopodia, and their cellular environment using Lucifer Yellow (LY) injections (followed by an anti-LY antibody), and the I-5 monoclonal antibody which reveals the peripheral axonal pathways (Ho and Goodman, Nature, 1982).

A few motoneurons pioneer peripheral axonal pathways very early in development. For example, the SETi growth cone pioneers nerve 3 and takes a stereotyped route through the coxa th the femur using unknown guidance cues. Most motoneurons follow peripheral axonal pathways. For example, the FETi growth cone follows nerve 5 into the limb bud and makes a series of cell-specific choices when confronted with alternative axonal pathways (flexor tibiae and tarsal motoneurons take different pathways when confronted with the same choices). These results suggest that the peripheral axonal pathways are labeled (just as we hypothesize pathways are labeled in the CNS, Raper, Bastiani, and Goodman, J. Neurosci., 1982a,b) and motoneuron growth cones are programmed to follow particular labels. The FETi growth cone finally arrives at the mesodermal mass giving rise to the extensor tibiae (ETi) muscle before the differentiating of muscle fibers begins. The FETi axon begins to extend thin branches into the mass of small mesodermal cells. At this stage, the mesodermal cells are organized around large muscle bundle pioneers that occur periodically where each of the many mature muscle fiber bundles will develop.

The muscle pioneers (Ball, Ho, Kotrla, and Goodman, this volume) may play two different roles as they guide motoneuron growth cones off of the peripheral axonal pathways. First, in several cases they serve as substrates for the navigation of identified growth cones to more distal muscle pioneers. Second, in other cases they appear to serve as cell-specific signals that stop growth cone extension. These results suggest that the muscle pioneers may be differentially labeled, and that different motoneuron growth cones are programmed to distinguish between a subset of labeled muscle pioneers. [Supported by the McKnight Foundation, NSF, and the Australian Natl. Univ.]

266.9 MORPHOGENESIS AND NAVIGATION OF PIONEER NEURON GROWTH CONES ALONG THE "GUIDEPOST" CELL CHAIN DURING GRASSHOPPER LIMB DEVELOPMENT.
David Bentley, Michael Caudy, Norman Herterich* and Dawn Yokoe*.
Zoology Dept. and Biophysics Group, Univ. California, Berkeley, CA 94720

The first pair of neurons (Til) to arise in the grasshopper leg project axons to the CNS along a chain of three cells or cell-pairs (designated F1, F2, and CT1 in distal to proximal order; Keshishian et al, this volume). Using an antibody technique (Caudy & Bentley, this volume), we examined about 600 Til pathways in the pro-, meso-, and metathoracic legs of 150 embryos at 28% to 38% of development. Til cells usually begin to stain as a tightly apposed pair within the epithelium (whence they arise), but twice stained before division when a single mother cell spanned the epithelium at the limb tip. CT1, F2, and F1 usually do not stain until filopodia are projected, although CT1 twice stained with a major cytoplasmic extension in the epithelium, suggesting epithelial origin. Each cell can stain before contact with Til growth cones, but F2 and F1 usually do not stain until later. The normal sequence of binding site acquisition is Til, CT1, F2, F1, and this order is also seen for an initial, radial, extension of filopodia (from each cell) and then for axonogenesis. Til growth cones always emerged from the "poles" of the cells (the cleavage plane between the cells is usually perpendicular to the limb axis, and this alignment is maintained). While growth cones normally emerge from proximal poles, they sometimes arise at both, or, in two cases, only distally. Since other emergence sites were never observed, internal cell structure may be important in determining the initial site of emergence. All cells in the chain display a similar sequence of early morphogenetic events, which may be relatively dependent on intrinsic factors.

The Til growth cone navigates across the inner surface of the epithelium, and generally has several major branches converging into a flattened axon (Later, the axon loses filopodia, rounds up, and is displaced from the epithelium.) Extending from the branches and the axon are many filopodia, which can be branched and can reach at least 100 microns; most project along the epithelium surface, but some reach between the cells almost to the limb surface. Early Til axons are usually in contact; occasionally, they follow divergent courses between the soma and F1, F1 and F2, or F2 and CT1, and then converge on the next "guidepost cell"(when crossing the cells, they may flatten, suggesting adhesion). Til filopodia can touch CT1 before the growth cone turns toward CT1. More often, they first contact distal CT1 filopodia (also the case for F2 filopodia). In rare instances, Til growth cones reach CT1 cells before they start axonogenesis. CT1 axons usually reach the CNS before efferent growth cones emerge, but occasionally efferent axons extend half way to CT1 cells which have not commenced axonogenesis. Although the relative positions of the cells in this chain are constant, their absolute positions vary substantially, e.g. F1 can be as close as one cell diameter to Til, or as far away as five cell diameters. Despite this variation, Til axons always grow from cell to cell along the chain.

266.10 IN VIVO ANTIBODY STAINING AND SELECTIVE KILLING OF IDENTIFIED "GUIDEPOST" NEURONS IN CULTURED GRASSHOPPER EMBRYOS.
Michael Caudy and David Bentley. Biophysics Group and Zoology Department, University of California, Berkeley, CA 94720

The first pair of afferent "pioneer" neurons to arise in embryonic grasshopper limbs projects axons from cell to cell along a chain of "guidepost" neurons whose placement may constitute the pathway to the CNS (Keshishian, et al, this volume). This hypothesis could be tested by selective elimination of guidepost cells. We report here a technique, comprised of double antibody labeling, in vivo staining, and UV irradiation, which has allowed individual guidepost cells to be located and killed in cultured embryos.

Anti-peroxidase antibody stains the surface of grasshopper neurons(Jan & Jan, PNAS 79: 2700, 1982). This staining can be greatly intensified: embryos were first exposed to rabbit anti-HRP primary antibody, then non-specific sites were quenched by rinsing in normal goat serum, and finally, fluorescein-conjugated goat anti-rabbit secondary antibodies were applied.This makes it possible to visualize the full array of filopodia from pioneer growth cones. Moreover, surface labeling displays morphological features, such as changes in axon diameter on different substrates (Bentley et al, this volume) without the osmotic artifacts of intracellular dye injections.

Surface labeling can be done in vivo: the dorsal closure of 30% stage embryos was opened, and the embryos were cultured in yolk-free saline-sucrose medium, with appropriate antibodies added. They were maintained at 30°C, in hanging drops, on an orbital shaker(50Hz). Both pairs of pioneers present in the limb at this stage, the tip cells (Ti1) and the cells to which they will project (CT1), stained brightly. Using image intensification, it should be possible to observe growth cone navigation. Embryos were cultured with antibody for the period during which the growth cones traverse the entire path from soma to CNS.

Identified "guidepost" neurons (CT1), located by in vivo double-staining, were selectively killed by irradiation: using a Leitz 50X water immersion Fluoreszenz lens (Numerical aperature: 1.0), a UV beam (390 nm cut-off) was reduced by a diaphram to the diameter of the targeted cell, and focused on the nucleus. Cell death was diagnosed by (1) changes in morphology (roughening of the cell membrane; darkening; agglutination of particles within the nucleus; swelling); (2) green-to-yellow autofluorescence(characteristic of normal cell death); (3) selective uptake of Trypan-blue dye (characteristic of cell death in many systems). In neuroblasts, 60 seconds exposure to UV was sufficient to initiate this response. Damage, judged by these criteria, could be confined to a single cell within an epithelium. CT1 neurons, stained in live embryos, were exposed to 15 min. of UV and displayed all three characteristics.

Using this technique, it should be possible to assess the role of guidepost cells in pathfinding by pioneer growth cones.

266.11 THE EFFECT OF MOTONEURON REMOVAL ON SENSORY NEURON OUTGROWTH IN CHICK HINDLIMB. Lynn Landmesser and Marcia Honig. Dept. of Biology, Yale University, New Haven, CT 06520 and Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

Sensory afferents form a precise and orderly segmental pattern of projections to the various muscle and cutaneous nerves of the chick hindlimb, which is correct from the onset of axonal growth into the limb (Honig, 1982, J. Physiol.). Further, after removal of a few segments of neural tube (and neural crest), afferents in the remaining segments still project along the appropriate nerves (Honig, 1979, Neurosci. Abstr. 5:163). Two mechanisms consistent with these findings might explain how sensory afferents establish the appropriate projection patterns. One involves the prespecification of afferents and their directed outgrowth in the limb. Alternatively, some afferents may associate with motoneuron axons, which grow out concurrently, and be guided to muscles; the remaining afferents would then project along cutaneous nerves. To test this second mechanism, we have examined the outgrowth of sensory afferents in the absence of motoneuron axons. The region of neural tube that contributes spinal nerves to the crural plexus was removed at St. 18-19, before motoneuron axons enter the limb, leaving the forming dorsal root ganglia intact. Embryos were allowed to develop until St. 30-32, 1-2 days after distinct peripheral nerves can first be distinguished in normal limbs.

In some of the 14 experimental limbs, several of the muscle nerves originating from the crural plexus were entirely missing. The other muscle nerves were often severely reduced in diameter, beyond the extent expected from the simple removal of their normal motoneuronal contribution. These results suggest that an interaction with motoneurons is necessary if sensory neurons are to project along motor pathways. It is likely that the relatively few motoneurons which were not removed by the operations can account for the formation of muscle nerves in the remaining cases.

An additional finding was that one or both of the two cutaneous nerves arising from the crural plexus was considerably enlarged in diameter in most of the experimental limbs as compared to unoperated animals. This suggests that afferents that might otherwise grow along muscle nerve pathways seem to, in the absence of motoneurons, project along cutaneous nerves. However, the presence of a few remaining motoneurons may be significant and these motoneurons may play an essential role in sensory neuron outgrowth. In addition, the possibility that some sensory neurons might, at even earlier stages in these experimental embryos, send processes along muscle nerve pathways must be examined. Nevertheless, these results clearly indicate that the presence of motoneurons is necessary for the establishment of appropriate sensory neuronal pathways within the limb. (Supported by NIH grants NS10666 (L.L.) and NS06427 (M.H.).

266.12 ASTROCYTIC MATURATION IN THE DEVELOPING CORPUS CALLOSUM OF THE RAT. K.L. Valentino and E.G. Jones. James L. O'Leary Division of Experimental Neurology and Neurological Surgery and McDonnell Center for the Study of Higher Brain Functions, Washington University School of Medicine, St. Louis, MO 63110

The differentiation of the interhemispheric region through which axons of the corpus callosum grow was studied using an antiserum to glial fibrillary acidic protein (GFAP), which is found in mature astrocytes (Eng et al., 1971, Brain Res. 28: 351) in developing astrocytes and in radial glia (Levitt et al., 1981, J. Neurosci. 1: 27). Rat fetuses of known gestational ages were removed from the mothers and perfused with either buffered 1% paraformaldehyde and 1% glutaraldehyde or 0.1M lysine, 0.01M sodium periodate and 0.1% paraformaldehyde. Brains were removed, frozen and serially sectioned through the corpus callosum in the horizontal and frontal planes on a cryostat. Sections were reacted for GFAP using either indirect immunofluorescence or the PAP technique.

Axons of the corpus callosum cross the midline on E18.5 (Valentino and Jones, J. Neurocytol., in press). On the 18th and 19th days of gestation, most of the GFAP in the cerebral hemispheres was contained in radial glial processes. There was intense staining of glial endfeet beneath the pial surface and at the medial walls of the lateral ventricles and some staining of cell bodies in the ventricular zone. At the region of interhemispheric fusion, the accumulation of astrocytic processes demonstrable electron microscopically showed only light immunochemical staining. These and a few that were darkly stained and presumably more mature, did not adopt any particular orientation. Though a few of the astrocyte processes were aligned along the path of the axons, many more were seen running rostro-caudally, radially, and dorso-ventrally. The overall impression as one moved towards the midline was of radially disposed astrocyte processes being disrupted and disoriented by the growing callosal axons and the fusion of the hemispheres. At no time could any orderly arrangement of GFAP containing processes be seen which might indicate that the processes were serving to guide the growing axons across the midline. There was no immunoreactive staining of cell bodies or processes ventral to the corpus callosum except in postnatal animals. This was associated with the developing cavum septi pellucidi and not with the formation of the genu of the corpus callosum. We conclude that orderly, morphological arrangements of astrocyte processes do not play an important role in the guidance of growing callosal axons.

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267.1 DORSAL MIDBRAIN SODIUM CURRENTS ARE REQUIRED FOR THE LORDOSIS REFLEX IN FEMALE RATS. R. E. Harlan*, B. D. Shivers and D. W. Pfaff (SPON: L.-M. Kow). The Rockefeller Univ., New York, NY 10021.

The lordosis reflex in female rats is estrogen-dependent and is elicited by somatosensory input from the flanks and perineum. Estrogen acts on cells in the mediobasal hypothalamus to induce and maintain lordotic responsiveness. Estrogen-modulated hypothalamic outflow to the dorsal midbrain participates in triggering the reflex. The present study was designed to investigate the requirement for voltage-dependent sodium currents in the dorsal midbrain for this behavior.

Ovariectomized, estrogen-treated rats bearing preimplanted intracranial cannulae were infused (1 µl bilaterally) while conscious with procaine (PC), tetrodotoxin (TTX) or vehicle and were tested for lordotic responsiveness by manual stimulation at the following intervals: 2, 5, 10, 20 and 40 min and 1, 2, 4, 8 and 12 h, and then daily for 7 days. Infusion of 50% PC (N=7) decreased lordotic responsiveness between 2 and 10 min post-infusion. Lordosis reflex scores (ranging from 0 = no lordosis to 3 = strongest possible lordosis) fell from 2.94±.06 (SEM) before infusion to 1.8±.1 2 min after PC infusion. Infusion of TTX (3.3 or 10 ng/µl; N=5 and 8) decreased lordotic responsiveness between 5 min and 8-12 h post-infusion. Lordosis reflex scores fell from pre-infusion levels of 3.0±0 to 0.96±.4 (3.3 ng TTX) and 1.4±.2 (10 ng TTX) 5 min post-infusion, and continued to drop until minimal scores were reached at 20 min (for 3.3 ng TTX, score = 0.68± .3) or 60 min (for 10 ng TTX, score = 0.50±.2) post-infusion. Infusion of either PC or TTX eliminated within 2 min audible vocalizations produced by most rats during manual stimulation tests. In 6/13 TTX-infused rats (0/7 PC-infused rats), the righting reflex disappeared, usually 40 min after infusion. Vehicle infusions (N=9) did not disrupt lordotic responsiveness, audible vocalizations or the righting reflex.

These results demonstrate that voltage-dependent sodium currents in the dorsal midbrain are required for the lordosis reflex. In the mediobasal hypothalamus, PC infusion had no effect and TTX infusion did not decrease lordotic responsiveness until 40 min post-infusion (Harlan, et al. Soc. Neurosci. Abstracts 7:615, 1982). The rapidity of the decrease in lordotic responsiveness following midbrain infusions of PC or TTX suggests that the effect is not simply due to disruption of outflow from the hypothalamus, but rather to loss of action potentials in dorsal midbrain neuronal elements. (Supported by HD 05585, HD 05737)

267.2 MIDBRAIN MICROINFUSIONS OF PROLACTIN FACILITATE THE LORDOSIS REFLEX OF FEMALE RATS. B. D. Shivers, R. E. Harlan* and D. W. Pfaff. The Rockefeller University, New York, NY 10021.

Medial hypothalamic and dorsal midbrain neurons control the appearance of an estrogen-dependent reproductive behavior, the lordosis reflex. Previous work shows that cells in the mediobasal hypothalamus containing prolactin-like immunoreactivity send fibers to the midbrain central gray (MCG). The purpose of the present study was to determine if prolactin microinfusions into the MCG facilitate the lordosis reflex in ovariectomized, estrogen-treated rats.

Ovariectomized rats bearing cannulae implanted in the MCG were injected sc with estrogen followed by progesterone, and tested manually to establish their ability to show strong lordosis reflexes. A week later, the rats received estrogen treatment resulting in the display of weak lordosis reflexes. On test days, the rats were lightly restrained, microinfused with test substances or vehicle, and tested manually for the lordosis reflex at the following intervals: 2, 5, 10, 20 and 40 min and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 9 and 24 h post-infusion. Microinfusions (1 µl bilaterally) of rat prolactin (NIAMDD-rPRL-B-3) into the MCG (400, 200 or 50 ng/µl; N=5,8 and 3) facilitated the lordosis reflex from 40 min to 4-8 h post-infusion. Microinfusions of vehicle (phosphate-buffered saline; N=7) or contaminants of rat prolactin (25 ng/µl growth hormone, NIAMDD-rGH-B-6, N=4; 1 ng/µl arginine vasopressin, Bachem, N=4) did not facilitate this behavior. Microinfusions of prolactin (N=5) into the MCG of ovariectomized, non-estrogen-treated rats also were not effective. Microinfusions of prolactin antiserum (NIAMDD; N=5) into the MCG of ovariectomized, estrogen-treated rats displaying strong lordosis reflexes inhibited the lordosis reflex from 10 min to 2 h post-infusion. Microinfusions of normal rabbit serum (Pentex, N=4) into the MCG did not inhibit this response.

A separate immunocytochemical study confirmed the presence of prolactin-like immunoreactivity in cells in the mediobasal hypothalamus and in fibers in the MCG.

The present results underscore the importance of the dorsal midbrain in modulating the lordosis reflex and suggest that a substance important for lordosis may be released in the midbrain, sharing antigenic determinants and structural features with prolactin. (Supported by HD 05737 and HD 05585)

267.3 POTENTIATION OF LORDOSIS BEHAVIOR BY PITUITARY-INACTIVE FRAGMENTS OF LUTEINIZING HORMONE-RELEASING HORMONE, C.A. Dudley and R.L. Moss. Dept. of Physiology, Univ. of Texas Health Science Center, Dallas, TX 75235.

Analogs of luteinizing hormone-releasing hormone (LHRH) have been reported to enhance lordotic responding in female rats and to influence the firing rate of individual hypothalamic neurons. These results, obtained by structurally modifying the LHRH molecule, lead to the hypothesis that presence of the entire molecule may not be required to potentiate sexual behavior. The present study was designed to investigate the effect of various inactive fragments of LHRH, which exert no effect on LH release from the pituitary gland, on lordotic responding in ovariectomized female rats.

Animals implanted with 23 gauge stainless steel cannula positioned in the third ventricle were primed with 0.2 mg estrone at 0 hrs., infused with 1 µl of one of three LHRH fragments dissolved in saline (100 ng/µl) or infused with saline at 46.5 hrs., and tested for sexual receptivity at 48 hrs. The following fragments, synthesized by W. Vale and J. Rivier, were tested: $LRF^{1-6}NH_2$, Ac-LRF^{5-10}, and des-Tyr^5LRF. Each fragment was tested in a separate group of animals and saline and drug treatments were counterbalanced. Standard tests for sexual receptivity were conducted, results were expressed in terms of the lordosis to mount (L/M) ratio, and statistical evaluations were made using matched-pair t or Student's t.

The results as seen in the table below indicate that $LRF^{1-6}NH_2$ had no effect on lordotic behavior. However, Ac-LRF^{5-10} and des-Tyr^5LRF produced a significant facilitatory effect on lordotic behavior. The results demonstrate that fragments of the LHRH molecule which are inactive at the pituitary level are capable of enhancing lordotic behavior. Furthermore, the data suggest that the last portion of the molecule is involved since residues 1-6 had no effect whereas residues 5-10 were active. Removal of Tyr^5 from the decapeptide did not interfere with behavioral activity, indicating that residues in positions 6-10 alone may be capable of facilitating lordotic behavior. This possibility will be explored in future research.

FRAGMENT	FRAGMENT: x̄ L/M	(n)	SALINE: x̄ L/M	(n)	t	p
$LRF^{1-6}NH_2$	.47	(15)	.44	(16)	0.25	ns
Ac-LRF^{5-10}	.76	(18)	.43	(19)	3.27	<.005
Ac-LRF^{5-10}	.80	(20)	.29	(20)	9.40	<.005
des-Tyr^5LRF	.80	(17)	.25	(17)	8.22	<.005

(Supported by NIH Grant #HD-11814).

267.4 β-ENDORPHIN SUPPRESSION OF MATING BEHAVIOR AND PLASMA LUTEINIZING HORMONE IN THE FEMALE RAT. J.B. Wiesner* and R.L. Moss. Dept. of Physiology, Univ. of Texas Hlth. Sci. Ctr., Dallas, TX 75235.

Various laboratories have reported that sexual behavior of male rats is suppressed by opiates and enhanced by the opioid antagonist naloxone (NAL). Since the neural substrate controlling sexual behavior in female rats differs from that in male rats, the effects of β-endorphin (βE) administration and opiate blockade on female receptivity were investigated.

Sexually experienced ovariectomized Sprague-Dawley rats implanted with 3rd ventricular cannulas were primed with estrogen (E) at 0 hrs and progesterone (P) at 24 hrs. At 30-34 hrs they received intraventricular infusions of either βE (100 ng) or saline vehicle (1 µl). The animals all received both treatments, one week apart, in counterbalanced fashion. None of the animals exhibited a cataleptic response to βE administration. Sexual receptivity, as measured by lordosis-to-mount ratio, was suppressed 15 min ($p<.05$) and 45 min ($p<.01$) after βE administration, after which it returned to control levels. In the second series of experiments, E-primed females received a subcutaneous injection of either NAL (2 mg/kg) or saline vehicle (0.1 cc), and mated 15, 45, 75 and 135 min after injection. All animals received both treatments, two weeks apart, in counterbalanced fashion. This dose of NAL had no significant effect upon receptivity. The animals were then tested similarly with 30 mg/kg NAL, which yielded slight but non-significant enhancement of mating behavior.

As a comparative study, NAL and various doses of βE were tested for their effects on secretion of LH. Ovariectomized rats which had been implanted with third ventricular cannulas were fitted with silastic jugular catheters for obtaining blood samples. 0.5 ml samples were taken before and 15, 30, 60 and 90 min after intraventricular infusion of either βE (doses ranging from 10 ng to 4 µg) or saline. LH was measured by RIA. βE significantly suppressed plasma LH at doses ≥ 50 ng ($p<.05$). In a separate experiment, ovariectomized rats with indwelling jugular catheters were given subcutaneous injections of NAL (2 mg/kg) or saline, and blood was sampled as above. NAL increased plasma LH levels at 15 min ($p<.005$) and 30 min ($p<.025$) after injection.

These results indicate that endogenous opioid peptides are capable of suppressing mating behavior in female rats in a subcataleptic dose range similar to that which suppresses plasma LH levels suggesting a possible role of endogenous opiates in the regulation of lordosis behavior. On the other hand, the failure of NAL to enhance lordosis behavior in a dose which is capable of increasing LH levels indicates that tonic opioid suppression of lordosis behavior is absent under the conditions tested, or that the opioid suppression is mediated via NAL-insensitive receptors.

(Supported by NIH Grant #HD-11814).

267.5 SEX DIFFERENCES IN THE PATTERN OF STEROID ACCUMULATION BY MOTONEURONS OF THE RAT LUMBAR SPINAL CORD. S.M.Breedlove and A.P.Arnold. Psychology Department, UCLA, Los Angeles, CA 90024.

We previously reported that male rat motoneurons accumulate radioactivity after injection of tritiated testosterone (T) or dihydrotestosterone (DHT), but not estradiol (E). We now report that lumbar motoneurons in male rats accumulate T or its metabolites more frequently than do female motoneurons. However, this sex difference in hormone accumulation by motoneurons is not observed following the injection of DHT or E, both of which are normal metabolites of T. The sex difference in hormone accumulation after injections of T but not DHT or E suggests that either there is a sex difference in the number of receptors for T itself, or there is a sex difference in the activity of enzymes which metabolize T in the rat lumbar spinal cord.

Three days after gonadectomy and adrenalectomy, adult rats were intra-ventricularly injected with 1.2 nmol/100 g body weight of either T, DHT or E (tritium labeled, 123-160 Ci/mmol). One hour later the animals were sacrificed and the lumbar-sacral spinal cord processed for thaw-mount autoradiography. A Poisson model of the distribution of reduced silver grains (Arnold, J Comp Neurol 189 421, 1980) was used to decide whether a given motoneuronal nucleus was labeled at a 1% level of confidence. Each hormone was injected into 3 males and 3 females. From each rat, at least 50 motoneuronal nuclei from each of 2 motoneuronal populations in the 5th & 6th lumbar segments were analyzed: the retrodorsolateral nucleus (RDLN) and the dorsolateral nucleus (DLN).

1. Very few motoneurons from either population accumulated E or its metabolites. In fact, some motoneurons had fewer silver grains over their nuclei than would be expected by chance, suggesting that these cells not only failed to accumulate E or its metabolites, but actively or passively excluded them, from their nuclei.

2. Virtually every motoneuron of either sex accumulated hormone after DHT injection.

3. Following T injection, more motoneurons were labeled in males than in females ($p<.05$), indicating a sex difference in hormone accumulation.

Taken together, these results suggest either a) there are separate receptors for T and DHT, and motoneurons have more T, but equal numbers of DHT receptors in males than in females, or b) the motoneurons of male rats have greater access to systemically administered T or its metabolites than do those of females.

	DLN		RDLN		The percentage of
HORMONE-	T	DHT	T	DHT	motoneurons labeled
males -	77.4%	96.4%	71.9%	90.2%	after hormone
females-	27.7%	94.8%	39.1%	92.1%	injection.

Supported by NIH grants HD15021 and RR07009.

267.6 GONADAL HORMONES SYNERGISTICALLY ALTER MAO ACTIVITY IN SPECIFIC HYPOTHALAMIC NUCLEI. V. N. Luine and J.C. Rhodes . Rockefeller University, New York, NY 10021 [Supported by PHS HD12011]

Monoamines present in the preoptic-hypothalamic area have been implicated in regulation of gonadotropin secretion and sexual behavior. Recent studies show that monoamine levels and turnover rates are altered by estrogen and progesterone in hypothalamic nuclei which are important for hormone mediated events. We have assessed whether activity of MAO (Type A), degradatory enzyme for monoamines, is affected by gonadal hormones in a manner consistent with a role in hormone mediated events. Also investigated were Acetylcholine esterase (AChE), degradative enzyme for actylcholine and Glucose-6-phosphate dehydrogenase (G6PDH), regulatory enzyme of the pentose phosphate pathway.

Estradiol (E_2) silastics (1cm) implanted in ovariectomized rats for 3 days led to: decreased MAO activity in the periventricular area (PVE) of the hypothalamus (25%), arcuate-median eminence (A-ME) (60%) and lateral ventromedial nucleus (LVMN) (50%); increased AChE in bed nucleus of stria terminalis (40%) and increased G6PDH in A-ME (45%), PVE of preoptic area (65%) and pituitary (70%). No enzymatic changes were noted after estradiol treatment in the anterior hypothalamic, medial ventromedial or dorsomedial nuclei. When 3 days of E_2 treatment was followed by 200 µg of progesterone i.v. and females killed 1 hr later, progesterone did not further change activity of AChE or G6PDH; however, activity of MAO was rapidly increased by progesterone in the LVMN and A-ME to values found in untreated ovariectomized-adrenalectomized controls. Rapid in vivo progesterone effects on MAO are dependent on prior estrogen treatment since progesterone administration to ovariectomized-adrenalectomized rats did not alter MAO activity. The mechanism for estrogen-progesterone dependent increased MAO activity was investigated by administration of anisomycin, a protein synthesis inhibitor, to estrogen primed females 1/2 hr before progesterone administration. Anisomycin did not block the 100% increase in LVMN MAO activity of estrogen-progesterone treated vs estrogen treated females. A direct effect of progesterone on MAO is suggested because progesterone added in vitro (10-8M) to homogenates of the basomedial hypothalamus from estrogen treated rats increases MAO activity. Such in vitro effects on MAO were not observed unless in vivo estrogen pretreatment was given, nor were in vitro progesterone effects observed on G6PDH.

Effects of gonadal hormones on MAO activity are consistent with a role for this enzyme in hormone mediated events since similar brain localizations (A-ME and LVMN), hormonal specificities and temporal characteristics (chronic E_2 and acute progesterone) are found for hormone action on MAO activity as for hormone action on female sexual behavior and gonadotropin secretion.

267.7 ANDROGEN REGULATES AROMATASE ACTIVITY IN THE HYPOTHALAMUS-PREOPTIC AREA OF ADULT RAT BRAINS. C. E. Roselli*, W. E. Ellinwood* and J. A. Resko* (SPON: C. L. Bethea). Physiology Dept., Oregon Health Sciences University, Portland, OR 97201 and Reproductive Physiology, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Estrogen formed from testosterone and androstenedione in the brain is thought to be involved in the hormonal control of gonadotropin secretion and male sexual behavior in some mammalian species. To determine whether aromatization in rat brain is under hormonal control, we measured aromatase activity in tissue homogenates from animals subjected to various endocrine manipulations. A highly sensitive in vitro assay that measures the release of ^3H_2O from ^3H-1 β-androstenedione was used to assess aromatization. The ^3H_2O formation (aromatase activity) increased linearly with time and tissue concentration. The enzyme demonstrated a pH optimum of 7.4 and a Michaelis constant of 0.05 µM. We found the highest amount of activity in the amygdala (AMYG) of intact male rats, with intermediate levels in the hypothalamus-preoptic area (HPOA) and low amounts in the cerebral cortex (CTX). Castration produced a significant decrease in aromatase activity in the HPOA ($p < .001$), but not in the AMYG or CTX ($p > .20$). The decline in HPOA aromatase activity occurred within 7 days and remained constant for up to 21 days after castration. In male rats that were given testosterone replacement by Silastic implants, HPOA aromatase activity remained at or above intact levels. Female brains had significantly less aromatase activity than male brains ($p < .001$), and aromatase activity was not affected by ovariectomy ($p > .20$). These findings suggest that androgen regulates the formation of estrogen in a discrete region of the male rat brain. Supported by NIH Grant 1-PD-HD1198.

267.8 GONADAL STEROIDS MODULATE SEX DIFFERENCES IN AN ELECTRIC ORGAN DISCHARGE WAVEFORM. A.H. Bass and C.D. Hopkins. Dept. Ecol. Behav. Biol., Univ. Minnesota, Minneapolis; C.N.R.S., Gif/Yvette, France.

Field studies of the mormyrid electric fishes of Gabon, West Africa have shown that males and females of some species have Electric Organ Discharges (EOD) that differ in duration and waveform. We have now completed field studies showing that gonadal steroids can induce a mature male-like EOD in immature specimens and mature females of such species. For one, Brienomyrus brachyistius long biphasic (LBP), the male EOD has an average duration of 2.25 ms (n=6) and an average peak power frequency (PPW), as determined with Fourier analysis, of 397 Hz (n=6); immature specimens and females have an EOD with an average duration of 0.908 ms (n=25) and an average PPW of 925 Hz (n=6). For LBP:

1) 2.0-4.0 mg of 17a-methyl testosterone dissolved in 1.2 L. of stream water periodically at 24 or 48 hr. intervals, induced a male-like EOD in immature fish and females over a 2 week period. When testosterone treatment was removed by returning the fish to fresh water, the EOD reverted to the immature/female waveform. Controls maintained in stream water for similar time periods showed no change in the EOD waveform.

2) The testosterone response did not depend on the presence of intact gonads as castrated fish showed a similar response.

3) Testosterone can be metabolized to another androgen, 5a-dihydrotestosterone (DHT) or an estrogen, estradiol (cf. McEwen, Science 211,1303,1980). Additional fish received pellet implants of DHT or 17B-estradiol. Only DHT induced a response similar to testosterone. Estradiol had a weak effect. The data suggest the response was androgen specific.

In a closely related species, B. brachyistius biphasic (BP), for which males and females have similar EODs (average duration of 0.412 ms and PPW of 1848 Hz; n=17), testosterone addition to the water had no effect on the EOD. This suggests that testosterone modulation of the EOD waveform is specific to species with sex differences in the waveform.

We recorded the command signal, arising in the spinal electromotoneurons that excite the electric organ. For BP, LBP normal female and LBP testosterone treated female, the command, as in other known mormyrids, consisted of 3 spikes separated by about 1.0 ms. Testosterone appears to exert its effect distal to the central generator at the level of the peripheral electrocyte, the fundamental structural unit of the electric organ. Electrocyte morphology differs between BP and LBP.

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267.9 SEX DIFFERENCES IN RAT BRAIN ESTROGEN AND PROGESTIN RECEPTORS
B. Parsons, T.C. Rainbow and B.S. McEwen. The Rockefeller University, New York, NY 10021.

Estradiol (E_2) and progesterone have different behavioral and neuroendocrine actions in the male and female rat. These sex differences in steroid response may result from differences in the number of steroid receptors in specific brain nuclei. We have examined this possibility by measuring levels of estrogen and progestin receptors in 6 nuclei (medial preoptic nucleus, ventromedial nucleus, bed nucleus of the stria terminalis, arcuate nucleus, and periventricular region of the preoptic area and of anterior hypothalamic nucleus) which contain high concentrations of gonadal steroid receptors, and which may be involved in reproductive behavior and gonadotropin secretion.

Rats were ovariectomized or castrated at least one week prior to use. For progestin receptor measurements, all rats received E_2 benzoate (10µg for 3 days) to induce progestin receptors to maximal levels. Animals were perfused with 10% cold dimethylsulfoxide to minimize tissue damage caused by freezing. Brain nuclei were removed from frozen (300µ) brain sections using hollow steel cannulas, 500µ or 1000µ in diameter. Estrogen and progestin receptors in cytosol were measured in vitro using 1nM ^{3}H-E_2 or 0.4nM ^{3}H-R5020. Non-specific binding was measured by co-incubation with 1µM moxestrol or 0.1µM R5020. Steroid bound to receptors was separated from free steroid on Sephadex LH-20 columns. Protein in each sample ranged from 5-50µg, and did not differ in male and female samples. Results are expressed as fmoles steroid specifically bound/mg protein.

Although the general pattern of receptor distribution was the same between males and females, there were substantial quantitative sex differences in the receptor content of specific nuclei. The medial preoptic nucleus, which may be the target region for E_2-induced control of gonadotropin release, had less than half the number of estrogen receptors in males as in females (7 fm/mg vs 17 fm/mg). The ventromedial nucleus, which may be the principal site for the activation of feminine reproductive behavior by progesterone, had less than one third the number of progestin receptors in males as in females (8 fm/mg vs 25 fm/mg). The periventricular region of the preoptic area also showed a significantly lower level of progestin receptor in males (25 fm/mg vs 50 fm/mg). There were no other significant sex differences in estrogen or progesterone receptor content among the 6 nuclei studied.

Our results indicate that the relative insensitivity of male rats to feminine actions of gonadal steroid might result in part from a lack of receptors in highly localized brain regions. We suggest that sex differences in neural receptors for gonadal steroids could be a general consequence of sexual differentiation.
Supported by NS07080, NS17435 and a grant from Rockefeller Found.

267.10 THE NEUROPSYCHOLOGY OF TURNER'S SYNDROME. John D. E. Gabrieli*, Suzanne Corkin, and John D. Crawford*. (SPON: Edith V. Sullivan). Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139; and Endocrine Metabolic Unit, Mass. Gen. Hosp., Boston, MA 02114.

Patients with Turner's syndrome are phenotypic females with X chromosome monosomy or partial monosomy due to a structurally abnormal second sex chromosome. They are gonadally dysgenic, have nonfunctional ovaries, and cannot produce normal levels of sex steroids. Such patients provide an opportunity to explore the influence of sex steroids on the development of human brain function and behavior. To date, 8 patients with Turner's syndrome ($\bar{X}$ = 12.3 yr) and 8 sibling control subjects ($\bar{X}$ = 12.3 yr) have undergone extensive neuropsychological evaluation.

The results indicated that patients with Turner's syndrome were impaired on the performance, but not the verbal, subtests of the age-appropriate Wechsler Intelligence Scale. The patients also demonstrated deficits on tests of personal and extrapersonal spatial skills (Body Scheme Test, Locomotor Mazes Test, Space Relations Test of the Primary Mental Abilities Tests), long-term memory for nonverbal visual materials (Rey-Taylor Test 1-hour recall, Gollin Incomplete-Pictures Test 24-hour retest), and memory for musical material (Tonal Memory subtest of the Seashore Measures of Musical Talents). However, the patients showed no deficit on tests of auditory or visual memory for verbal material, tests of verbal production and problem solving, and several motor tasks.

The patients, 7 of whom were right handed, failed to exhibit a right-hand advantage on two tests of manual dexterity (Fine-Finger Movement Test and Grooved Pegboard Test).The siblings did show a right-hand superiority, even though 2 of them were not right handed. The siblings had a right-ear advantage on a verbal dichotic listening test and a left-hand superiority for tactile spatial perception; these laterality effects were less prevalent among patients.

The findings suggest that patients with Turner's syndrome have selective impairments in cognitive abilities for which the right hemisphere is thought to be specialized. Further, these patients show a less lateralized pattern of performance on perceptual tasks that reflect hemispheric specialization and on motor tasks that produce manual asymmetries in performance. Because patients with Turner's syndrome are underexposed to sex steroids from about the fourth month in utero, it may be that normal exposure to some or all sex steroids is necessary for the normal development of hemispheric specialization and for the full expression of higher cognitive skills subserved primarily by the right hemisphere.
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267.11 REVERSAL OF PHOTOPERIODIC ANESTRUS BY GROWTH-INDUCING LIMBIC LESIONS IN FEMALE HAMSTERS. J.Nichols* and K.T.Borer.(SPON: J.M. Sprague) Department of Physical Education,University of Michigan, Ann Arbor, MI 48109.

Voluntary running activity can reverse photoperiodic anestrus in female hamsters maintained in short-day (SD) photoperiod (10L:14D) and is accompanied by increases in serum and pituitary growth hormone (GH) and prolactin (PRL) concentrations (Borer,K.T. et al.,11th Annual Meeting,Soc.Neurosci.,1981,Abs.70.14). Administration of GH stimulates testicular growth and reproduction in male hamsters maintained in SD photoperiod (Bex,F.et al.,Endocrinology 103:2069,1978). We have,therefore, examined whether the reversal of photoperiodic anestrus was a consequence of exercise-induced increases in GH concentration and in somatic growth in hamsters maintained in SD photoperiod.

Rapid growth was induced by bilateral transections of dorsal hippocampus (HIPPO,n=9,Borer,K.T.et al.,Neuroendocrinology 29: 22,1979) or electrolytic lesions of rostromedial septum (SEP,n=7, Borer,K.T.et al.,Neuroendocrinology 23:133,1977) in anestrous female hamsters maintained in SD photoperiod (8L:16D).

Measurements were made of: (1)ponderal growth rate (daily), (2)body fat content (day 16), (3)serum GH and PRL concentrations with respective homologous RIAs (day 16),(4)total level and pattern of running activity in wheels (days 14-15), and (5) vaginal secretions (daily).

We found: (1) a 14 to 18-fold increase in the rate of ponderal growth ($p < 0.01$) following an initial weight loss (2 days in SEP, 7 days in HIPPO,1 day in control hamsters), (2) no change in body fat content, (3) increases in serum concentrations of GH (SEP: 184.7±46.9, HIPPO: 73.2±23.5, controls: 24.3±8.0 ng/ml,$p \leq 0.05$) and PRL (SEP: 29.1±7.1, HIPPO: 6.9±1.2, controls: 5.2 ±0.5. $p < 0.01$),(4)significant reduction in total activity level but no change in the time of onset of running activity, and (5) increased incidence in estrous cycling (3 SEP,7 HIPPO,1 control,$p < 0.01$) in limbic-lesioned relative to sham-operated hamsters (n=7).

We conclude that: (1) acceleration of growth and increases in serum GH concentration are not the probable cause for the reversal of photoperiodic anestrus in exercising hamsters, and that (2) variable damage to septo-hippocampal fibers most probably accounts for variable reinstatement of estrus in hamsters maintained in SD photoperiod.

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267.12 ANTERIOR PITUITARY IMPLANTS, o-PROLACTIN AND ESTRADIOL TREATMENTS INCREASE STRIATAL DOPAMINE RECEPTOR DENSITY IN INTACT, CASTRATED AND HYPOPHYSECTOMIZED RATS. T. Di Paolo, P. Poyet* and F. Labrie*. Department of Molecular Endocrinology, Le Centre Hospitalier de l'Université Laval, Québec G1V 4G2, Canada.

We have shown that chronic estradiol treatment increases the density of striatal dopamine receptors in ovariectomized rats (Mol. Cell. Endocr. 16: 99, 1979). Since estrogen treatment increases circulating prolactin levels, we have investigated the possibility that prolactin exerts a stimulatory effect on striatal dopamine receptors. Ovariectomized female or intact male rats were implanted with 3 adenohypophyses under the kidney capsule or treated with 17β-estradiol (10 µg, b.i.d.) for 2 weeks. In rats of both sexes, the pituitary implants and the estradiol treatment increased [^{3}H]spiperone binding to striatal dopamine receptors (males + implants: 132% vs control, $p < 0.01$; males + estradiol: 123% vs control, $p < 0.05$; ovariectomized females + implants: 135% vs control, $p < 0.01$; ovariectomized females + estradiol: 120% vs control, $p < 0.05$). This effect of estradiol and pituitary implants on dopamine receptors was further investigated in ovariectomized rats; these animals had elevated plasma prolactin levels and increased density of striatal dopamine receptors without alteration of their affinity. We next investigated the possible indirect action of estradiol via prolactin in ovariectomized and hypophysectomized female rats treated with 17β-estradiol (10 µg, b.i.d.) or bearing 3 anterior pituitary implants for 2 weeks. The pituitary implants elevated blood prolactin levels in hypophysectomized and ovariectomized animals while estradiol elevated prolactin levels only in ovariectomized rats. Both estradiol or prolactin from the implants elevated striatal [^{3}H]spiperone binding in hypophysectomized and also in ovariectomized rats. However, hypophysectomized animals had about 20% less binding capacity than ovariectomized rats. Hypophysectomy and prolactin treatment was further studied in hypophysectomized female rats treated with ovine prolactin (500 µg, b.i.d.) or 17β-estradiol (10 µg, b.i.d.) for 5 days. Prolactin or estradiol treatments increase striatal dopamine receptor density of hypophysectomized rats compared to hypophysectomized controls. However, hypophysectomy itself significantly decreased the striatal dopamine receptor density (25%) compared to intact or ovariectomized female rats. These results indicate that high prolactin levels induced by pituitary implants or by injections of ovine prolactin lead, as does chronic estradiol treatment, to an increased density of striatal dopamine receptors. However, the effect of estradiol may not be explained exclusively by increased prolactin levels since this supersensitivity is also observed in hypophysectomized rats. Our data indicate that the pituitary may have its own modulating influence on striatal dopamine receptors.

268.1 ORIGIN, PRENATAL DEVELOPMENT & STRUCTURAL ORGANIZATION OF LAYER I OF HUMAN CEREBRAL CORTEX. A GOLGI STUDY. M. Marin-Padilla and T.M. Marin-Padilla*. Department of Pathology, Dartmouth Medical School, Hanover, N.H. 03755.

The origin and entire prenatal development of layer I of the human cerebral (motor) cortex has been investigated with the rapid Golgi method. The cases studied include the following developmental stages: 7,11,15,18,20,24,26,29,30,32,36,38 and 40 weeks of gestation respectively. The components of layer I have been separated into essential and non-essential, intrinsic and extrinsic elements. The specific afferents of layer I (primitive corticipetal fibers) and the Cajal-Retzius neurons are considered to be the essential intrinsic components of this superficial lamina; the apical dendritic bouquets of all pyramidal neurons and the axonic terminations of all Martinotti neurons are considered to be its essential extrinsic components; and, the small neurons of layer I and the terminal fibrils from thalamic, callosal and cortico-cortical afferent systems are considered to be its non-essential intrinsic and extrinsic components respectively. The appearance, structure and prenatal development of each one of the components of layer I will be analyzed and discussed. In addition, the structural interrelationships among the various components of layer I during the course of prenatal cortical ontogenesis will be illustrated with camera lucida drawings. (Supported by the National Institute of Child Health and Human Development, grant # 09274).

268.2 AFFERENT CONNECTIONS OF THE PRIMATE INFERIOR ARCUATE CORTEX. T.W. Deacon*, P. Rosenberg*, M.K. Eckert*, and C.E. Shank*. (SPON: W.B. Forbes). Biol. Anthro. Lab., Harvard Univ., Cambridge, MA 02138.

The origins of afferents to the cortex on either bank of the inferior limb of the arcuate sulcus in the frontal lobe of macaque monkeys (Macaca fascicularis) were investigated using horseradish peroxidase (HRP), HRP conjugated with wheat germ agglutinin, and the fluorescent tracer bisbenzimide.

Injections confined to the posterior bank of the inferior arcuate sulcus (area 44) labeled ipsilateral cortical cells in prefrontal cortex lateral to the principal sulcus but not extending into its banks; orbital cortex lateral to medial orbital sulcus near the pole; the fundus and both banks of the cingulate sulcus, occuring in distinct clumps in its anterior half; medial premotor (supplementary motor) area; dorsal bank of the insula; inferior parietal cortex (area 7) lateral to the inferior parietal sulcus; and posterior superior temporal cortex; a few labeled cells were also observed in the dorsal bank of the superior temporal sulcus midway down the temporal lobe and in lateral premotor cortex. Contralaterally, labeled cells were found only in homotopic periarcuate cortex. Without exception cortical cells were found in middle layers, II through IV, never in deep layers. Thalamic cells were labeled in the inferior and lateral parts of the medial dorsal nucleus, medial pulvinar, central dorsal, central lateral, and limitans nuclei. Other subcortical sites containing a few labeled cells include the nucleus of the diagonal band of Broca and the substantia innominata.

An injection just anterior to the inferior arcuate sulcus at the same level (dorsal area 45) involving the area just lateral to the frontal eye fields, exhibited an entirely different pattern of afferents. Labeled cells were found in cortical area 8 just dorsal and anterior to the injection site: contralateral homotopic cortex; periarcuate cortex inferiorly; the ventral bank of the superior temporal sulcus; the inferior temporal cortex between the inferior occipital and occipito-temporal sulci; and more posteriorly in the dorsal bank of the inferior occipital sulcus. Thalamic labeling was observed only in the lateral portion of the medial dorsal nucleus.

The posterior bank of the inferior arcuate sulcus is in the unique position of receiving afferents from cortical and subcortical areas known to support vocalization in monkeys (1) and from parietal and temporal association cortices and supplementary motor cortex which are considered homologues of the "language" areas in humans (2).

1. Robinson (1967) Physiol. Behav. 2:345-354.
2. Galaburda & Pandya (1982) In: Primate Brain Evolution.

268.3 CYTOARCHITECTURE AND INTRINSIC CONNECTIONS OF THE PREFRONTAL CORTEX OF THE RHESUS MONKEY. H. Barbas and D. N. Pandya. Dept. Health Sci., Boston Univ., and V.A. Medical Center, Bedford, MA.

Cytoarchitectonic analysis of the prefrontal cortex reveals two sites with a bilaminar periallocortex, bounded by proisocortical areas with a rudimentary laminar organization. One of these periallo-proisocortices is situated at the rostral tip of the cingulate sulcus on the medial surface, and the other is in the caudal portion of the orbital surface. From each of these periallo-proisocortical areas, a stepwise laminar differentiation can be identified. From the cingulate proisocortex successive regions with increasing granularity in layer IV can be traced dorsally through areas 9, 10, 46 and 8. Likewise, from the orbital proisocortex, a similar progressive laminar differentiation can be followed ventrally towards areas 13, 10, 12, 46 and 8.

Intrinsic connections of subdivisions of the prefrontal cortex, studied with the anterograde transport of tritiated amino acids, support the above cytoarchitectonic trends. For example, the frontal cingulate proisocortex projects to the medial periallocortical area, on one hand, and to the dorsomedial isocortical areas 9 and 10 on the other. Likewise, the cortex above the middle portion of the principal sulcus (area 46) projects rostro-dorsally to the less granular areas 9 and 10, and caudally to the more granular area 8 in the arcuate concavity. Similar patterns were also observed for regions below the principal sulcus. Thus, the orbital area 13 projects to the periallocortical and proisocortical regions on the orbital surface and to the surrounding isocortical areas 14, 12, 11 and 10. Ventral area 46 projects rostro-ventrally to areas 12, 11 and 10, and caudally to ventral area 8. The latter, along with dorsal area 8, shows the highest degree of laminar differentiation within the prefrontal cortex. The projection zone of area 8 remains restricted to the immediate isocortical regions. Although the basic projection patterns of subregions of the prefrontal cortex follow either a ventral or a dorsal course, there is evidence for reciprocal connections between dorsal and ventral regions which have similar cytoarchitectural features.

Thus the cytoarchitecture of the prefrontal cortex shows two distinct trends which may be correlated with the connectional patterns. Moreover, within each trend, a given cortical region projects to an architectonically less differentiated area, as well as to a region with a more developed cortical laminar organization.

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268.4 DISTRIBUTION OF ACETYLCHOLINESTERASE-REACTIVE CELL BODIES AND FIBRES IN THE FRONTAL GRANULAR CORTEX OF THE HUMAN BRAIN. I. Kostović. Department of Anatomy, Medical Faculty, University of Zagreb, 41001 Zagreb, Yugoslavia.

Intense acetylcholinesterase /AChE/ reactivity appears to be remarkably accurate marker for the distribution of cholinergic elements in the frontal cortex and related basal telencephalic nuclei /Johnston et al.,Exp.Brain Res.,43:159, 1981/. In this study we have analyzed distribution of strongly AChE-reactive fibres and cell bodies in the frontal granular cortex of the human brain. The blocks of human lateral prefrontal cortex /area frontalis granularis and frontopolaris of Economo and Koskinas/ were obtained as autopsy material within 8 hours after death. Frozen sections were treated by modification of Koelle's method for demonstration of AChE activity and Nissl method for cytoarchitectonic analysis. There are two striking features which characterize AChE reactivity of the frontal cortex: a/ rich network of AChE--reactive fibres and b/ presence of strongly AChE-reactive pyramidal cell layer within the deep part of cortical lamina III. AChE-reactive fibrillar network seems to originate in the white matter, especially in the external capsule. In the layer VI AChE-reactive fibres show predominantely oblique orientation. Layer V contains thick fibres covered by short spiny projections. Layer IV is characterized by very fine caliber of fibres and diffuse reactivity. In layers III and II fibres form plexiform neuropil. Zone of the most superficial fibrillar staining is concentrated in the deeper half of layer I, where many fibres run in a tangential direction. AChE-reactive cell bodies were observed throughout all cortical layers. However, the vast majority of strongly reactive cell bodies was found in layers III C and VI. In layer III C AChE-reactive cell bodies have pyramidal shape with clear outlines of apical and basal processes and variable amount of AChE-reactive granular product. AChE-reactive cells of layer III C tend to be arranged in clusters. AChE-reactive cell bodies of layer VI are polymorph /multipolar and fusiform/. The polymorph AChE-reactive neurons were also found below layer VI, within the white matter. The present histochemical data suggest that human prefrontal cortex is densely innervated by putative cholinergic /strongly AChE--reactive/ extrinsic fibres that show tangential and plexiform pattern of distribution, without obvious radial compartmentalization. In addition, human prefrontal cortex contains significant population of intrinsic, strongly AChE-reactive neurons. Pyramidal AChE-reactive neurons show cluster phenomenon.

/Supported by PL 480/

268.5 CENTRAL CONNECTIONS OF THE VIBRISSAL REGION OF MOUSE PRIMARY MOTOR CORTEX. L.L. Porter* and E.L. White
(SPON. M. Feldman). Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA. 02118.

The afferent and efferent connections of the vibrissal representation within the mouse primary motor cortex (MsI) were identified using the retrograde transport of horseradish peroxidase (HRP) and the anterograde transport of tritiated amino acids injected into MsI. Following aldehyde perfusion brains were frozen sectioned at 40 um and reacted for HRP using the 3-3' diaminobenzidine-colbalt chloride technique of Adams ('77). Alternate HRP reacted sections were processed for autoradiography.

HRP-filled pyramidal cell somata and concentrations of developed silver grains above background levels were observed in both the vibrissal area of SmI cortex (ie. the Posteromedial Barrel Subfield; PMBSF cortex) and in the face region of SmII (area 40). In both regions labeled somata occurred predominately in cortical layers II-III and V. Autoradiographic label was superimposed over the regions containing labeled somata but exhibited a less distinct laminar organization than that of the labeled neurons.

A dense reciprocal projection connected the injection site with the homotopic area in contralateral MsI; somata occurred for the most part in layer V, but many were found in layer III. Developed silver grains were uniformly dispersed over the area containing labeled cell bodies. HRP labeled pyramidal somata were noted in contralateral PMBSF cortex, but no silver grains occurred in this region.

Reciprocal projections linked MsI cortex with the ipsilateral thalamic nuclei: ventralis pars lateralis (VL) and centralis pars lateralis (CL) and with the zona incerta (ZI). Labeled cell bodies and developed silver grains were more dense in VL than in other thalamic nuclei. The ipsilateral striatum and thalamic reticular nucleus (NRT) received afferents from the motor cortex but did not project to it.

The vibrissal area of primary motor cortex is thus connected with a number of cortical and subcortical structures, each of which has been shown to play a role in motor performance.
Supported by NSF grant BNS 8202614

268.6 AFFERENT CONNECTIONS OF AREA 7 IN THE CAT. C.R. Olson* and K.A. Lawler* (SPON: B. Sakitt). Dept. Psych., MIT, Cambridge, MA 02139.

Area 7, a zone located on the crown of the cat's suprasylvian gyrus, is thought to be homologous to the primate area of the same name, and thus to be involved in sensorimotor integration. The purpose of the experiments here described was to identify brain structures projecting to area 7 in the cat and to establish the topographic pattern of the connections. We placed small deposits of distinguishable retrograde tracers (NY,Bb and HRP) into different parts of area 7, allowed two days' survival, and then prepared the brains for charting of transported label. The results demonstrate a surprisingly clear pattern of spatial organization in pathways linking area 7 to other neural association centers.

Labeled neurons were present in many cortical areas including the frontal eye fields and cingulate cortex. In the medial frontal eye field, on the lip of the cruciate sulcus, the location of labeled cells was displaced medially as the injection site in area 7 was moved rostrally. In the lateral field, topographic organization also was evident, but labeling generally was weaker. The posterior cingulate gyrus and adjacent ventral bank of the splenial sulcus contained numerous heavily labeled neurons in all cases. The labeled neurons usually formed a narrow curved band stretching forward and upward along the cingulate gyrus and into the splenial sulcus. This band was displaced rostrally and ventrally when tracer was injected more anteriorly in area 7. Other cortical association zones exhibiting weak to moderate labeling included the anterior suprasylvian sulcus, posterior ectosylvian gyrus, orbital gyrus and caudal perirhinal cortex.

Heavy labeling of subcortical neurons occurred only in the thalamus and the claustrum. Specific thalamic nuclei projecting in a topographically organized pattern to area 7 included the pulvinar and the caudal division of the lateral intermediate nucleus. Of nonspecific nuclei, the central lateral nucleus in particular contained labeled neurons. Cells projecting to different parts of area 7 were homogeneously intermingled in this zone. Labeling in the claustrum was restricted to a division immediately underlying regions linked to visual and somatosensory cortex. The pathway linking this part of the claustrum to area 7 exhibited clear topographic organization.

Taken together, our results suggest that there is regional specialization of function within area 7 and brain regions to which it is linked. This pattern of specialization is preserved by topographically organized interconnecting pathways.
Supported by Johnson & Johnson postdoctoral fellowship(C.R.O.), Fight for Sight postdoctoral fellowship(K.A.L.) and NIH 5R01-EY-02866 & 5 P30-EY-02621 (Dr. Ann M. Graybiel).

268.7 REGIONAL (^{14}C) 2-DEOXYGLUCOSE UPTAKE DURING FORELIMB MOVEMENTS EVOKED BY MOTOR CORTEX STIMULATION. F.R. Sharp and K.L. Evans*. Dept. of Neurosciences, UCSD School of Medicine, La Jolla, California 92093

Repetitive left forelimb movements were produced in adult, alert rats by electrically stimulating right motor cortex. Regions of increased (^{14}C) 2-deoxyglucose (2DG) uptake were mapped autoradiographically with structure boundaries identified from Nissl stains.

2DG uptake increased in right motor cortex about the electrode and in a separate anterior region which could represent the rat second forelimb motor region, MII. 2DG uptake increased in two separate columnar regions of somatosensory cortex, possibly representing forelimb SmI and SmII. Subcortical regions which increased 2DG uptake ipsilateral to cortical stimulation included: anterior dorsolateral caudate-putamen; lateral globus pallidus; posterior entopeduncular nucleus; ventral reticular N. thalamus; ventral ventrolateral N. thalamus; ventral ventrobasal N. thalamus; posteriomedial and parafasicular N. thalamus; subthalamic N.; posterior ventrolateral substantia nigra pars reticulata; ventral red nucleus pars magnocellularis; nucleus cuneiformis; deep mesencephalic nucleus; deep layers of superior colliculus; medial and ventral pontine nuclei; inferior olive; and perhaps N. reticularis tegmenti pontis. Contralateral subcortical 2DG uptake increases occurred in: lateral and interpositus n. cerebellum; cerebellar hemisphere regions lobulus simplex, crus I, crus II, and paramedian lobule; lateral reticular N.; n. cuneatus of the dorsal columns; intermediate layers of cervical cord gray matter; and occasionally triceps and wrist extensor muscles. The 2DG uptake in cerebellar hemisphere increased primarily in the granule cell layer and occurred in discrete microzones in the paramedian lobule.

When the above results are compared to the regions activated during vibrissae motor cortex stimulation, almost all subcortical regions are somatotopically organized. In addition, several structures are activated during forelimb stimulation which were not activated during vibrissae stimulation including ventrobasal n., inferior olive, interpositus N. cerebellum, lateral reticular nucleus, n. cuneatus, cervical cord, and forelimb muscles.

268.8 CORTICO-STRIATO-PALLIDAL SYSTEMS: HOW MANY ARE THERE? J.H. Fallon & S.E. Loughlin. Dept. Anat. Univ. of Calif. Irvine, CA 92717.

The extrapyramidal motor system is dominated by the massive funneling of descending connections in the neocortically-originating cortico-striato-pallidal system ("Neo-CSP"). Heimer and colleagues (1975,1978,1982) extended the CSP concept to the basal forebrain. These allocortically-arising CSP systems ("Allo-CSP") in ventral forebrain regions would, thus, parallel the dorsal systems. Our recent anatomical and histochemical findings in the albino rat (Fallon, 1981; 1982; Fallon et al., 1982a; 1982b; Ribak and Fallon, 1982) suggested that a third CSP system exists in the medial sectors of each Allo-CSP system (e.g., olfactory tubercle, amygdala, nucleus accumbens, and septum). These medial areas concentrate steroids such as estrogens, receive poorly collateralized dopaminergic inputs from the VTA, and contain a unique combination of neuroactive peptides such as the enkephalins, substance P and releasing hormones, which indicate that these areas are part of an endocrine CSP system ("Endo-CSP").

In the present study, we have examined the connections and neurotransmitter relationships of the ventral basal forebrain (olfactory tubercle area) of the albino rat. Anterograde and retrograde connections were determined in 83 rats with HRP or the fluorescent retrograde tracers Nuclear Yellow, Propidium Iodide, and True Blue. The distribution of neuroactive peptides was determined by immunofluorescence of met and leu-enkephalin, substance P, LHRH and CCK (antibodies from Immunonuclear Corporation) in 42 rats. Estradiol binding was determined by ^{3}H-estradiol autoradiography in 15 rats.

The results of these anatomical studies indicate that neurons of the medial and lateral areas of the basal forebrain regions (e.g., olfactory tubercle) have similar afferent and efferent connections which qualify them as CSP systems, but differ in other characteristics. For example, medial areas contain denser concentrations of the peptides and more cells concentrate estradiol, and the dopamine cells innervating medial sectors of forebrain nuclei arise from the poorly collateralized neurons of the ventral tegmental area and not the highly collateralized neurons of the adjacent substantia nigra (Fallon, 1981). We, thus, propose the notion of multiple CSP systems. Given the medial-lateral gradient of peptide distribution in the CSP systems, and a topographical organization of ascending and descending connections in the CSP systems probably related to specific sensory, limbic and endocrine "motor" loops, each funneled "cone" of descending CSP activity may form a "preferred channel" of extrapyramidal efferent activity modulated by local GABA inhibitory projections to adjacent channels at each horizontal level of the CSP. The concept of CSP systems as an organizational scheme in motor systems might, thus, be extended to be a more general scheme for organization of a multiplicity of brain systems. Therefore, there may be CSP systems orders of magnitude more numerous than initially envisioned.

This study is supported by NIH Grant NS 16017.

268.9 AFFERENT CONNECTIONS OF THE PERIRHINAL CORTEX (AREA 35) IN THE RAT: AN AUTORADIOGRAPHIC AND HORSERADISH PEROXIDASE TRACER STUDY. H. Eichenbaum, T.W. Deacon*, P. Rosenberg* and K. Eckmann*. Dept. Biol., Wellesley Coll., Wellesley, MA 02181; Dept. Anthro., Harvard Univ., Cambridge, MA 02138.

Afferent connections of the perirhinal cortex in the rat brain were studied using anterograde and retrograde tracers. Ipsilateral cortical afferents to this cortex originate predominately from cells in layer II of dorso-medial frontal, frontal-sulcal, and ventral lateral parietal cortex (area 13) including the sulcal cortex immediately anterior to the perirhinal cortex; predominately from middle and deep layers of lateral parietal cortex (area 7) and posterior lateral cortex (areas 36 and lateral 18); and from the cingulate cortex, field CA1 of the hippocampus, subicular areas, and the diagonal band. Subcortical projections originate in the claustrum, the lateral nucleus of the amygdala, the nucleus reuniens of the thalamus, the mammillary complex of the hypothalamus, and the median raphe nuclei. Contralateral projections originate from cells in the dorso-medial frontal, cingulate, subicular, and homotopic contralateral perirhinal cortex.

Neocortical perirhinal afferents demonstrate a rostral-caudal topography. Rostrally it receives afferents predominantly from the rostral half of the neocortex, including dorso-medial frontal, sulcal frontal, lateral insular, and parietal cortex, and does not receive hippocampal or subicular afferents. More caudally it receives few if any frontal afferents, but does receive parietal, cingulate, and posterior lateral cortex afferents, as well as afferents from subicular areas and CA1. N. reuniens constitutes the only source of thalamic afferents to both subdivisions. The region immediately posterior to rhinal sulcus cortex is distinguished by a lack of neocortical afferents and by afferents originating in the N. anterior dorsalis and N. ventralis medialis as well as from N. reuniens in the thalamus. It also receives projections from subicular areas and CA1.

There is also a distinct dorsal-ventral topography. The dorsal lateral entorhinal area receives afferents from the nucleus reuniens of the thalamus, claustrum, and diagonal band; but also from the olfactory bulbs, piriform cortex, cortico-basal amygdala, peri-amygdaloid cortex, and not from neocortical areas above the rhinal sulcus. Area 36, just dorsal to the rhinal sulcus, receives afferents from the dorsally adjacent cortical areas, the adjacent perirhinal cortex, and the medial geniculate nucleus of the thalamus. Thus, it appears that neocortical-limbic projections do not traverse the rhinal sulcus on their way to the hippocampal system in the rat, but rather all share a final common relay in the posterior rhinal sulcus.

268.10 LAMINAR DISTRIBUTION AND DENSITY OF CORTICAL NEURONS WITH CALLOSAL AND ASSOCIATIONAL COLLATERALS IN LIMBIC AND ASSOCIATION CORTEX OF THE DEVELOPING RHESUS MONKEY. M.L. Schwartz and P.S. Goldman-Rakic, Sec. of Neuroanatomy, Yale Univ. School of Medicine, New Haven, CT 06510.

Using double labeling with fluorescent tracers, we have recently described a small population of neurons in the limbic and association cortex of adult rhesus monkeys which have both "callosal" and "associational" axons (Schwartz and Goldman-Rakic, Neurosci. Abst., 7, 417, 1981). The present study was undertaken to examine the relative number and laminar distribution of such neurons during ontogenetic development. Our analysis is based on data from six rhesus monkeys divided among three age groups: two fetuses (embryonic ages E132 and E137), two neonatal (4 and 12 days of postnatal age) and two adult rhesus monkeys. Based on previous autoradiographic studies the injections in fetuses were made one month before birth at about the time that cortical axons penetrate the cortical plate in this species (Goldman-Rakic, The Organization of Cerebral Cortex, F.O. Schmitt, Ed., MIT Press, 1981, pp. 69-97). Each animal was injected with either propidium iodide, fast blue or nuclear yellow into the dorsal bank of the principal sulcus (PS) of the left hemisphere, followed by injection of a different dye into the intraparietal sulcus (IPS) of the right hemisphere. The size and sulcal landmarks of the monkey brain permitted precise localization of injections in the same cytoarchitectonic areas at each age. Following appropriate survival periods, the monkeys were sacrificed and the brain tissue prepared for fluorescence microscopy.

In the two fetuses, the homotopic cortical areas contralateral to the PS and IPS injection sites as well as the cingulate cortex of both hemispheres contained large numbers of single labeled neurons, confirming that both callosal and associational connections are established in rhesus monkey at least one month before birth. The density, laminar distribution and typology of single labeled neurons in fetuses was similar to that in neonatal and adult monkeys. The fetal cortex also contained double labeled neurons, that is, cells with axons in both hemispheres. As in the adult, these were primarily medium sized pyramidal cells found exclusively in layer III. Most importantly, the percentage of double labeled cells was about 1%, a value which corresponds to their incidence in the mature rhesus monkey. The present findings indicate that in primates, neurons with divergent axon collaterals in selected areas of association and limbic cortex belong to a class whose number and connections remain stable throughout development.

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268.11 SIMULTANEOUS RECORDINGS OF SINGLE UNIT ACTIVITY IN CORTEX AND THALAMUS DURING RECRUITING RESPONSES INDUCED BY REPETITIVE STIMULATION OF NUCLEUS CENTRALIS MEDIALIS. M. Avoli Montreal Neurological Institute & Department of Neurology & Neurosurgery, McGill University, Montréal, Canada H3A 2B4.

In 7 cats extracellular single unit recordings were performed simultaneously from pairs of neurons, one located in the cortex (areas 5 and 7), the other in the thalamus (n.lateralis posterior and pulvinar). The activity of these units was studied during recruiting responses (RR) induced by trains (duration: 0.8-2sec) of stimuli (4-10Hz, 0.1-0.3 msec, 0.5-2mA) delivered to n. centralis medialis. The EEG was recorded with the cortical and thalamic microelectrodes. Some of the thalamic units were identified by cortical stimulation. A computer generated post-stimulus time histograms (PSTH) of unit activity and averages of EEG waves.

The PSTHs of cortical unit activity displayed an increase of firing probability in association with the surface negative cortical wave of RR (latency: 25-40 msec). The cortical firing, which progressively increased during a train of stimuli, was followed by a period of "inhibition" which appeared to be directly related to the degree of the preceding excitation and was at times associated with a positive wave in the depth of the cortex. Two main types of thalamic units could be differentiated with respect to their behavior during RR. The first type fired earlier than the simultaneously recorded cortical units. Two of these thalamic units were identified as thalamocortical. The second type of thalamic units was silenced during the period of cortical firing, but tended to fire action potentials during the period of cortical "inhibition". Such a reciprocal relationship between cortex and thalamus was only seen during RR, whilst single shock stimulation of n. centralis medialis did not appear to induce such behavior.

These data demonstrate that thalamic neurons located in the so-called specific nuclei of the thalamus (e.g. LP-pulvinar) are recruited during repetitive stimulation of midline thalamic structures (so-called unspecific nuclei). The temporal relationship between the firing of cortical and thalamic units suggests that cortical RR, at least in the cat association system are dependent upon thalamocortical volleys generated by LP-pulvinar neurons.

268.12 ELECTROPHYSIOLOGICAL ACTIVITY RECORDED FROM THE CEREBRAL CORTEX OF AN ISOLATED MAMMALIAN BRAIN MAINTAINED IN VITRO. K. Walton and R. Llinas. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York 10016.

An in vitro mammalian (guinea pig) brain preparation has been developed. This is an extension of the isolated cerebellum-brain stem introduced in 1980 (Llinas, Yarom & Sugimori, Soc. Neurosci. Abst., 6:513, 1980). The preparation comprises the entire brain from the level of C_1 and includes the olfactory bulbs. The brain is perfused through one vertebral artery, the other vertebral and both internal carotid arteries being tied off. The perfusate is a Krebs saline modified by the addition of polyvinylpyrrolidone 40 (to achieve a correct osmolarity) and .003% hydrogen peroxide (for increased oxygenation). The preparation is superfused with a similar saline solution and maintained at 35 to 37°C. Anatomical studies following introduction of dye via the perfusion system indicate that, under these conditions, perfusate reaches the entire brain and that the integrity of the blood-brain barrier is maintained for at least 5 hrs.

Viability has been tested electrophysiologically in the cerebellar, visual and olfactory cortices. In the olfactory system, following orthodromic stimulation via the lateral olfactory tract (LOT), evoked potentials have been recorded from the prepyriform cortex (PPC) and olfactory tubercle. The waveform recorded from the surface of the PPC resembles that recorded from the cat in vivo (Biedenbach & Stevens, J. Neurophysiol., 32:193, 1969) and from slices of guinea pig cortex (Richards & Sercombe, J. Physiol. 197, 1968). After the stimulus artifact, a small wave, marking the synchronous LOT volley is followed by a large amplitude negative wave. This response has the same duration as in cat (about 20 ms) but is more complex, exhibiting superimposed sharp positive peaks. Finally, a long (70 ms) positive wave is seen. As the LOT stimulus is increased, a more complex, polyphasic waveform is recorded. This also has been reported in the cat in vivo (Freeman, J. Neurophysiol., 31:337, 1968). As the microelectrode is advanced into the cortex, the surface-negative component of the waveform decreases in amplitude and inverts (300-350 microns). The surface-positive wave reverses between 350-400 microns. This pattern of reversal resembles that seen in cat in vivo.

These preliminary recordings, together with those made in the visual cortex (following direct cortical stimulation), indicate that synaptic activity is present, even in rostral cortical regions, in the mammalian brain maintained under conditions of complete sensory and circulatory isolation.

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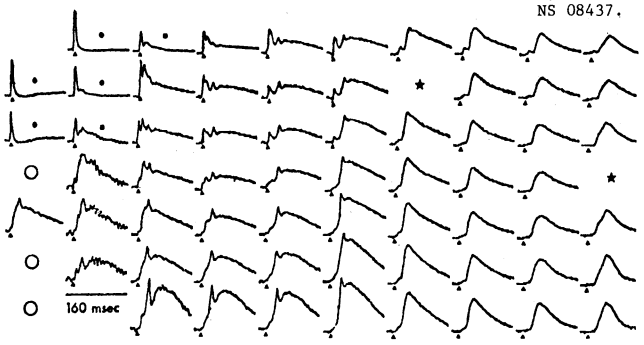
268.13 SIMULTANEOUS OPTICAL MONITORING OF ACTIVITY FROM MANY AREAS OF THE SALAMANDER OLFACTORY BULB; A NEW METHOD FOR STUDYING FUNCTIONAL ORGANIZATION IN THE VERTEBRATE CNS. H.S. Orbach and L.B. Cohen. Dept. of Physiol., Yale Univ. Sch. of Med., New Haven, CT. 06510.

We investigated the use of voltage sensitive dyes in the salamander (Ambystoma tigrinum) olfactory bulb with the hope of developing a new technique for studying functional organization. The optical measurements were made by forming a magnified image of the stained bulb on a 124 element photodiode array. The largest signals have come from absorption measurements with transmitted light using merocyanine dyes or fluorescence measurements with epi-illumination using styryl dyes. The figure illustrates the output of the array elements from a single trial measurement of absorption in an in vitro preparation. The olfactory nerve, entering from the upper left was stimulated via a suction electrode at the time marked by the triangle on each trace. The wavelength dependence of the signal and the effects of 10 μM TTX showed that the signals were not movement or stimulus artifacts. In many experiments, we did not detect pharmacological effects or photodynamic damage due to the dye. We think that the fast, short latency peak may be the action potentials in the incoming olfactory axons; the second peak occurs at the time of the mitral cell bursts. Peaks with similar delays were also seen in simultaneous electrode measurements of field potentials. There are also long-lasting signals which arise after a substantial delay. In this experiment a 7X objective was used; each element of the array received light from an area on the bulb of 200 μm x 200 μm. Our in vivo measurements have not been substantially noisier. We are optimistic that in vivo measurements in mammals will be possible using the epi-fluorescence method. Supported by N.I.H. grant NS 08437.



269.1 VESTIBULAR NERVE FUNCTION AFTER IPSILATERAL LABYRINTHECTOMY. David W. Jensen, Dept. of Oto/Com. Sci. and Program in Neuroscience, Baylor College of Medicine, Houston, Texas, 77030.

Morphological data in studies from cats, squirrel monkeys, and humans have shown that the vestibular nerve survives after it has been deafferentated. Given this, it is of interest to know (1) What functional capabilities if any remain in the nerve? and (2) If any function does remain, what behavioral significance might it have?

Guinea pigs were used. Electro-physiological recordings were taken of discharge activity of the superior vestibular ganglion cells, and of post-synaptic responses of vestibular nuclear cells to electrical stimulation of the ipsilateral vestibular nerve. 6 were intact, and 5 animals had received 1-6 weeks beforehand, a left labyrinthectomy. In addition to the labyrinthectomy, peripheral axotomies of all the branchlets of the superior branch, plus the saccular branchlet of the nerve, were performed under direct visual control. Deafferentation of the posterior canal nerve was done by blind probing and scraping of the posterior ampullary recess using a fine pick.

In the intact superior ganglion, 93 cells had a mean discharge rate of 42 ($\pm$20 S.D.) imp/sec. Also, electrical stimulation of the nerve evoked monosynaptic responses in the ipsilateral vestibular nuclei. An additional presynaptic waveform was clearly visible when the electrode tip was near the vestibular nerve root. In the deafferentated superior ganglion, spontaneous spike activity was held long enough for computer analysis in every preparation examined at 5, 7, 12, 39 and 55 days post op. However, the mean number of computer analyzable cells per electrode penetration was only 0.7 ($\pm$1.7 S.D.) compared to 3.3 ($\pm$3.3 S.D.) for the intact ganglion. But, if injury discharges are counted, it is 2.2 ($\pm$2.3 S.D.) versus 4.0 ($\pm$3.4 S.D.) cells/penetration respectively. The mean discharge rate for the deafferentated cells was 5.4 ($\pm$6.1 S.D.) imp/sec (n=16). Electrical stimulation of the deafferentated superior branch stump evoked a slow phase-type eye movement, and pre and post monosynaptic-type responses in the ipsilateral vestibular nuclei. Finally, after compensation had taken place in two animals, the left nerve stump and ganglion were removed. Both animals then exhibited marked leftward postural asymmetries and a slight right beating eye nystagmus.

In conclusion, after deafferentation of the vestibular nerve, a retention and/or a regeneration of nerve function takes place, and this function appears to play a role in maintaining postural balance.

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269.2 REGULAR AND IRREGULAR ACTIVITY RECORDED FROM PRIMARY AFFERENTS OF THE HORIZONTAL SEMICIRCULAR CANAL OF THE LIZARD CALOTES VERSICOLOR. MORPHOLOGICAL AND PHYSIOLOGICAL CONSIDERATIONS. D.A. Schessel* and S.M. Highstein (SPON: A. Mitsacos) Albert Einstein College of Medicine, Bronx, New York 10461.

In spinalized lizards spontaneous activity of primary vestibular afferents of the horizontal semicircular canal was recorded with horseradish peroxidase (HRP) loaded glass microelectrodes (Schessel and Highstein, Neuroscience Abs. 22.9, 1981). Coefficient of variation (c.v.= standard deviation/mean) was calculated and correlated with the morphological type of afferent terminal in the crista. Of 17 injected afferents, 12 had a c.v.>0.4 indicating a high degree of irregularity and all 12 demonstrated a multicalyx enwrapping 2-5 Type I hair cells. These multicalicies tended to be located in the central portions of the crista. Neurons with a c.v.<0.4 were more regular and could be innervated purely by type II hair cells at bouton-like endings or could be dimorphic terminals consisting of both calyx and bouton-like terminations. The largest diameter fibers (c.a. 10μ) tended to be multicalicies while dimorphic or bouton-like afferents had intermediate or small diameters. Chemical excitatory post synaptic potentials (EPSPs) were observed to underlie action potential (AP) initiation in afferents with multicalyx terminals while EPSPs were not visible or appeared as minute depolarizations in dimorphic or bouton-like afferents. The base line between APs in irregular (c.v.>0.4) afferents was flat except for EPSPs while in regular (c.v.<0.4) afferents a scoop-ramp membrane potential trajectory between APs was observed (Highstein & Politoff, Brain Res. 150, 1978). Assuming that recording sites are not far distant from impulse initiation sites, two mechanisms for AP initiation are suggested. In irregular fibers EPSPs underlie APs and the frequency and summation of these EPSPs controls the degree of regularity. In regular fibers APs are initiated by summated, smoothed EPSPs and the membrane potential between successive APs has a scoop-ramp appearance. We presume that regularity is conferred on dimorphic or bouton-like afferents by built in conductances which hyperpolarize the post spike membrane potential. As this hyperpolarization dies away, depolarization by summated EPSPs brings the membrane potential to threshold when a new AP is initiated. Thus we envision that the degree of regularity is conferred on vestibular primary afferents by both pre- and post-synaptic factors. Namely, the intensity of innervation and the electrotonic distance between EPSP initiation sites and impulse initiation sites and the absence or presence of post-spike conductances built into the afferent fibers.

Supported by NS 07512

269.3 COMPARISON OF RESPONSES OF VESTIBULAR AFFERENTS AND SECOND ORDER NEURONS TO SINUSOIDAL POLARIZATION. K. Ezure*, M.S. Cohen* and V.J. Wilson. Rockefeller University, N.Y., N.Y. 10021

We studied the response of cat semicircular canal afferents to sinusoidal polarizing currents applied to an electrode implanted in the horizontal ampulla. Activity was recorded, in decerebrate cats, from the rostral portion of the superior division of the vestibular nerve in the vicinity of Scarpa's ganglion. Electrode implantation abolished responses to natural stimulation and reduced the level of resting activity. The distribution of coefficients of variation of resting activity was similar to that seen with an intact labyrinth. Polarizing currents in the frequency range 0.175-4 Hz modulated the activity of many fibers sinusoidally. Responses were similar to those described for monkey afferents by Goldberg, Smith and Fernandez (Soc. Neurosci. Abst. 6: 224, 1980). Phase typically led stimulus negativity by approximately 14°, although half of the regular fibers had a phase lead that increased with frequency. Mean sensitivity (spikes/sec/μA) of regular and irregular fibers increased about 1.5 times over the frequency range studied: absolute sensitivity was about 7 times higher for irregular than for regular fibers. Afferent behavior could be well described by a transfer function with a fractional exponent. Response of afferent fibers to sinusoidal current was compared with that of second-order neurons studied earlier under similar conditions (e.g. V.J. Wilson et al., J. Neurophysiol. 42: 331-345, 1979). Slopes of the sensitivities were similar, but second order neurons developed a phase advance over afferents at frequencies around 1 Hz. This difference in dynamics can be described by a transfer function in the form $\tau s + 1$, with τ = 12 msec. This predicts a phase lead of second order neurons over afferents of 25° at 6 Hz, a frequency still of physiological interest. It remains to be determined whether this applies only to a subset of second-order neurons contributing to vestibulocollic reflexes. Supported by N.I.H. grants NS02619 and RR07065.

269.4 VESTIBULAR EFFECTS ON RENSHAW CELLS. H.-G. Ross, M. Thewissen*, S. Cleveland* and J. Purrmann*. Physiol. Institut, Univ. Düsseldorf, Fed. Rep. of Germany

We have investigated the effects of electrical stimulation of vestibular afferents on the antidromically induced burst responses of individual Renshaw cells in the lumbar spinal cord of decerebrate cats.

METHODS: Stimulating electrodes were implanted near individual vestibular nerve branches (Suzuki et al., Ann. Otol. St. Louis 78: 815-826 (1969)); appropriate location was checked by observing eye movements induced by stimulation during reduced anesthesia (N_2O-halothane). After laminectomy ipsilateral dorsal roots L6-S2 were cut and the following ipsilateral nerves were prepared for stimulation: gastrocnemius-soleus, deep peroneal, posterior biceps-semitendinosus. After intercollicular decerebration anesthesia was removed and the animals were paralyzed and artificially respired. Renshaw cells were recorded using conventional glass micropipettes filled with KCl. In each experiment the effect of vestibular stimulation on α-motoneuron excitability was also determined: in the nerves previously used for Renshaw cell activation a reflex discharge was elicited by single shocks to the appropriate dorsal root.

RESULTS: With ipsilateral vestibular stimulation (1o pulses at 5oo Hz), inhibition of Renshaw cell discharge--measured as the decrease in the number of spikes per burst--often reached a maximum of 6o to 7o %. The maximum inhibition usually occurred at a latency of 5o to 2oo ms between the beginning of vestibular stimulation and a single antidromic shock. Inhibition was always found, regardless of whether a cell was activated from a flexor or an extensor nerve and independently of whether the reflex motoneuron discharge in that nerve was facilitated or inhibited. All Renshaw cells thus appear to receive inhibition from the vestibular apparatus, which is in contrast to the differential effects on α-motoneurons. Owing to the spontaneous activity of the vestibular apparatus at rest, this of course includes the possibility of disinhibition leading to increased Renshaw cell excitability. Thus, the vestibular apparatus has the potential to exert powerful control over the gain of the motor output by modulating the effectiveness of recurrent inhibition.

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269.5 DYNAMICS OF HORIZONTAL NYSTAGMUS INDUCED BY VERTICAL STIMULATION WHILE ROTATING. T. Raphan, B. Cohen and V. Henn*. Dept. of Computer and Information Science, Brooklyn College, Brooklyn, N.Y. and Depts. of Neurology, Mt. Sinai School of Medicine, New York, N.Y. and Univ. of Zurich, Zurich, Switzerland.

Rotation about a vertical axis in darkness causes horizontal per-rotatory nystagmus for any angle of static tilt as long as there is a horizontal component of head rotation. If monkeys are pitched back 60° so that the lateral canals are perpendicular to the plane of rotation, a horizontal component is still induced. This activity must arise from the vertical canals. At each angle of tilt the nystagmus decays to zero as rotation continues. If animals are sinusoidally pitched forward and back in darkness without rotation, oscillating vertical nystagmus but no horizontal nystagmus is induced. However, if these stimuli are combined, that is, if the head is sinusoidally pitched with regard to gravity while animals are rotated about a vertical axis, continuous unidirectional horizontal nystagmus is produced. There is also sinusoidally varying vertical nystagmus as well as modulation of the steady state horizontal slow phase velocity at the pitch frequency. Whether the axis about which the animal is pitched is above or below the labyrinths is not significant in determining the steady state velocity. This makes it unlikely that a rotating linear force field produced by Coriolis and gravity forces is primarily responsible for generating the continuous horizontal nystagmus. Thus, the velocity signal responsible for the continuous nystagmus does not arise in the otolith organs. If pitching is begun during rotation in darkness, nystagmus builds slowly to a steady state level. If animals stop pitching but continue rotating, horizontal nystagmus decays back to zero. Both the rise and fall time constant of the nystagmus at the onset and end of pitch are approximately equal to that of per and post-rotatory nystagmus induced by rotation about a vertical axis without pitching and to optokinetic after-nystagmus (OKAN). This suggests that the velocity storage mechanism is activated by this stimulus. In accord with this, steady state eye velocity of the induced horizontal nystagmus increases approximately linearly with rotational velocity up to the saturation velocity of OKAN. Within the effective range it is independent of pitch frequency. Moreover, when rotation and pitch are halted, the post-rotatory nystagmus is reduced by the amount of the preceding steady state velocity. This study shows that dynamic head movements in a gravitational field during rotation can excite the semicircular canals to provide an estimate of head velocity. The velocity storage mechanism appears to play an important role in processing this information.
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269.6 ORIGIN OF LABYRINTHINE ACTIVITY RESPONSIBLE FOR NYSTAGMUS DURING PITCH WHILE ROTATING. B. Cohen, T. Raphan, J.I. Suzuki* and V. Henn*. Depts. of Neurology, Mt. Sinai School of Medicine, New York & University of Zurich, Switzerland, Dept. of Computer & Information Science, Brooklyn College and Dept. of Otolaryngology, Teikyo University, Tokyo, Japan.

Selective labyrinthine lesions were made in monkeys to determine the site of origin of activity and the central processing responsible for the continuous nystagmus during pitch while rotating. Semicircular canals were plugged by grinding across them with a fine diamond burr, or the lateral semicircular canal nerves were cut. After both lateral canals were plugged, horizontal OKN and OKAN were readily induced as was horizontal nystagmus induced by off vertical axis rotation (OVAR). Vertical vestibular nystagmus and OKN were intact. This shows that the vertical canals, the otolith organs and the velocity storage mechanism were functional. There was no response to rotation in the plane of the lateral canals in darkness. Despite this a component of horizontal nystagmus was readily elicited if animals were statically tilted up 60° and rotated with the lateral canals perpendicular to the plane of rotation. This indicates that the vertical canals can elicit a horizontal component of nystagmus. In accord with this, continuous horizontal nystagmus was induced by pitch during rotation in lateral canal-plugged animals. After the anterior and posterior canals were plugged, horizontal nystagmus was readily elicited by rotation in the plane of the lateral canals. Horizontal OKN and OKAN were normal as was the nystagmus induced by OVAR. However, there was no horizontal component of rotation when animals were statically tilted so that the lateral canals were perpendicular to the plane of rotation. Moreover, horizontal nystagmus was not induced by pitching while rotating. Thus, although the lateral canals and otolith organs were functional, the response to pitch while rotating was lost. After the lateral canal nerves were cut, horizontal OKAN was lost, indicating that the horizontal velocity storage mechanism was inactivated. Vertical OKN, OKAN and post-rotatory responses were present after recovery from surgery, and had gains that were close to normal. Despite the intact vertical semicircular canals, horizontal nystagmus could not be elicited by pitch during rotation after the storage integrator had been inactivated. These data support the hypothesis that activity responsible for generating the continuous horizontal nystagmus during pitch while rotating arises in the vertical semicircular canals and couples to the oculomotor system through the velocity storage integrator.
Supported by NS00294 and Academic Investigator Award EY00157 (TR).

269.7 IMPROVED VISUAL MODULATION OF VOR IN THE CAT, R.M. Douglas*, H. Flohr*, M.T. Feran* and G. Melvill Jones. (SPON: D. Watt), AMRU, Dept. of Physiol., McGill Univ., Montreal, PQ, Canada.

It is known that the dark-tested VOR gain (eye vel./head vel.) is adaptively reduced after prolonged exposure to rotation in the light with a visual field which is fixed to the turntable. This study examined whether other mechanisms besides VOR adaptation per se, can be invoked to cope with this sensory conflict situation. Four cats were sinusoidally rotated at 1/6 Hz and 50°/s amp whilst viewing the fixed field for 2 or 3 hrs per session. Sessions were repeated once a week for up to 10 weeks, normal vision and movement being allowed between sessions. VOR gain was measured (search coil method) by sampling every 5 to 10 min, both in the dark and while viewing the fixed screen (concentric striped cylinder). The fixed field, light tested, data differed considerably from expectation based on the dark tested VOR data. 1) Initial Values: VOR gain in the dark was around 0.94 before the first, and all other sessions. All cats had difficulty with the fixed field condition, the average gain during the first 10 cycles of the first session in the light being 0.75 (ideally it should be zero). However, for later sessions this initial value progressively decreased, eventually becoming as low as 0.3. In one cat the initial fixed field gain was still low (0.4) when measured 6 weeks after the previous (10th) session. 2) Time Constant: The dark tested VOR gain declined by about 45% during each session and the time constant of this decline remained essentially unchanged at about 50 min for all sessions. In contrast the fixed field gain dropped progressively faster from one session to the next. Initially the time constant of decline was about 50 min, as above, but eventually it dropped to less than 10 min (and became unmeasurable with the sampling intervals used). The fixed field gain at the end of the first 2 hr session was about 0.34 and this declined slightly with repetition to about 0.26 after 10 sessions. 3) Storage in the Dark: After some sessions cats were held overnight in complete darkness. Next day the dark tested gain was still subnormal, at about 0.74, representing about 44% retention of the previous day's adaptation. In contrast there was about 80% overnight retention of the previous day's fixed field gain attenuation. Conclusions: In addition to adaptive VOR gain changes, this form of visual-vestibular conflict produced more effective use of visual signals. OKN is unlikely to have been responsible since this influence tends to decrease with decrease of VOR gain. Therefore it appears that either the otherwise limited visual pursuit system of the cat, or some other, more subtle, form of visually induced VOR 'cancellation' or both, can be significantly enhanced. In any case this visual effect exhibits different properties from those of the dark-tested VOR adaptation.
Supported by NSERC and MRC of Canada.

269.8 HIGH FREQUENCY CHARACTERISTICS OF THE HUMAN VERTICAL VESTIBULO-OCULAR REFLEX DURING ACTIVE HEAD MOVEMENTS. J. H. Anderson and S. L. Liston*. Depts. of Otolaryngology and Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

The vertical vestibulo-ocular reflex (VOR) can help to maintain gaze during disturbances of head posture. Based on studies of horizontal eye movements it may have particular importance at the higher frequencies of head movements, wherein the semicircular canals are stimulated. The aim of the present work is to quantitate some of the response characteristics of the vertical VOR over the frequency range 0.2 - 3.0 Hz.

Human subjects were used. Each volunteer sat in a completely dark room and rotated his head up and down (from 10 to 30 deg) in synchrony with a sinusoidally modulated acoustic stimulus. EOG electrodes were used to record the vertical eye movements and a triaxial linear accelerometer, rigidly mounted to the subject's head, was used to measure head position relative to gravity. Prior to analysis, the fast phase portions of the eye movements were removed, digitally, and a cumulative slow phase eye position was calculated. Then a trend analysis, digital filtering, and Fourier analysis were performed on both the head and eye movements. Results from 4 subjects showed a small phase lead (0-10 deg) of eye position relative head position up to about 0.8 - 1.0 Hz. Above 1.0 Hz the phase showed a slight lag, 5-15 deg at 3.0 Hz. The gain (amplitude ratio) had values of 0.5 - 0.7 and was flat or showed a decrease above 1.0 Hz.

These results are similar to those obtained during active horizontal head movements (Tomlinson, R.D. et al., Acta Otolaryngol., 90:184, 1980), wherein the VOR gain was flat above 1.0 Hz, and are different from those obtained during passive horizontal rotation (Keller, E., Vis. Res., 18:311, 1978; Benson, A.J., Recent Adv. Aerospace Med., 249, 1970) where the horizontal VOR gain increases to values greater than 1.0 above 1.0 Hz. This may suggest an interaction of the vertical VOR with other inputs, e.g., neck proprioceptive, during fast head movements. Alternatively, the high frequency dynamics of the vertical VOR may be different. (Supported by USPHS grants NS12125 and NS16567.)

269.9 EFFECT OF LONG-TERM 2X MAGNIFIED VISUAL INPUT ON THE HUMAN VESTIBULO-OCULAR REFLEX. Y. Istl*, D. Hyden* and D.W.F. Schwarz. Lab of Otoneurology, University of Toronto, Toronto, Ontario, Canada.

Past studies which used dove-prism and telescopic lenses as a means of modifying visual input, revealed that the vestibulo-ocular reflex (VOR) undergoes a re-calibration to maintain stable vision. However, recent evidence has indicated that it is unsatisfactory to use low frequency sinusoidal stimulation for VOR measurements (Hyden et al., Acta Otolaryngol., in press). It has been demonstrated, that by applying frequencies above 2 Hz in a random fashion, the functional operation of the VOR can be studied without influence from the visuomotor system and central motor programmes. It was the objective of this study to examine the effectiveness of VOR adaptation when non-vestibular influences were excluded, thereby enabling us to determine if VOR adaptation is sufficient to account for the adaptive process or if other factors contribute to the recalibration process.

The horizontal eye movements of six healthy subjects were tested in total darkness using conventional EOG techniques before and during a 5 day period in which 2X telescopic spectacles were worn. Subjects were oscillated a) sinusoidally (0.5 and 3.0 Hz; constant P-P velocity 110°/s) and b) pseudo-randomly (0.5-5.0 Hz; maximum P-P velocity 110°/s) and were instructed to imagine either 1) an earth-fixed target or 2) a moving target.

Complete adaptation was achieved at 3 Hz with a sinusoidal stimulus (gain of 2). In contrast, an adaptation of only 70% was achieved at 3 Hz with random stimulation in the first visual paradigm in which subjects attempted to produce perfect compensatory eye movements and 50% with VOR suppression (second visual paradigm). When subjects were oscillated sinusoidally at 0.5 Hz while imagining an earth-fixed target, they attained a mean gain increase of only 61%. The restricted gain increase in the latter test condition may be attributable to the difficulty of the "fixation" task. During the adaptation period it was noted, that the saccadic system was involved in increasing the eye movement responses when the VOR had not reached the fully-adapted state. These saccades were edited out of the eye movement records before the gains were calculated.

In conclusion, our results suggest that the VOR is not exclusively involved in the adaptive process; rather, central motor programmes collaborate with the VOR to bring about complete adaptation.

Supported by the Medical Research Council of Canada.

269.10 BRAIN CATECHOLAMINES MODULATE VISUAL ADAPTABILITY OF THE VESTIBULOOCULAR REFLEX IN THE CAT. E.L. Keller and M.J. Smith*. Smith-Kettlewell Inst. of Visual Sciences, San Francisco, CA 94115

It has been shown in a variety of species that prism or lens alteration of the normal visual input leads to a rapid plastic adaptation of the vestibulooocular reflex (VOR). Another line of experiments has demonstrated that brain catecholamines (CAs) are involved in modulating the plastic adaptability of visual systems within the visual cortex. In the present study we investigated whether brain CAs might also be responsible for modulating the plasticity of the VOR.

Four cats were subjected to a five-hour period of forced head rotations while their vision was restricted to that seen through fixed field spectacles (+8.0 lens with the visual scene at one focal length and moving with the cat). This regime resulted in more than 50% mean reduction in VOR gain by the end of the adapting period. The same group of animals then received intracisternal injections of the CA-related neurotoxin, 6-hydroxydopamine (6-OHDA). Following a two-week period to permit large, 6-OHDA induced depletion of brain CAs, the cats were again tested for VOR adaptation. An identical five-hour period of visual adaptation now produced only a 16% mean reduction in gain. Furthermore the animal with the largest levels of CA depletion (as assayed subsequently on each animal by biochemical means) showed almost no adaptation.

The measured reductions in brain CAs, as tested in cortex and cerebellar samples, were accompanied by large increases above normal levels in medullary brain stem. Thus the observed suppression of VOR adaptation may have resulted alternatively from increased CAs in this region. In either case the data clearly show that brain CAs play a role in regulating neural plasticity in the VOR and support the hypothesis of their general role in controlling adaptive neural processes.

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269.11 ADAPTIVE PLASTICITY IN THE FLATFISH VESTIBULO-OCULAR REFLEX IS ACHIEVED BY REARRANGING SECONDARY VESTIBULAR NEURON CONNECTIONS TO OCULOMOTONEURONS. W. Graf and R. Baker. Rockefeller Univ., NY, NY 10021 and New York Univ. Med. Ctr., NY, NY 10016.

During metamorphosis all species of flatfish experience a 90° change in orientation between their vestibular and ocular coordinate axes. The situation arises because the body tilts 90° to one side and the eye which would have faced the sea bottom migrates around the dorsal aspect of the fish. As a result, the optic axes for both eyes maintain their orientation with respect to earth horizontal, but the horizontal semicircular canals (HC) become vertically oriented. Since the flatfish propels its body with the same swimming movements as a normal fish the HCs are still exposed to identical accelerations, which now occur in a vertical plane. The appropriate compensatory eye movements are simultaneous rotations of both eyes forward or backward (i.e. parallel), in contrast to the symmetric eye movements in upright fish (i.e. one eye moves forward, the other backward). Since reorganization of the vestibulo-ocular pathways must be hypothesized, we studied the location and termination of secondary vestibular neurons that were physiologically identified as being linked to the HC with intracellular HRP methods in Pseudopleuronectes americanus. Two distinct types of HC second order vestibular neurons were observed. In the first case, cells were located in the anterior vestibular nucleus. After crossing the midline the axon ascended in the medial longitudinal fasciculus (MLF). Major termination sites were in the inferior oblique and superior rectus subdivisions of the oculomotor nucleus. Axonal branches then recrossed the midline and terminated in identical locations on the contralateral side. In the second group, cells were located in the descending vestibular nucleus. Their axons crossed the midline and also ascended in the MLF. Major termination sites were in the trochlear nucleus and in the inferior rectus subdivision of the oculomotor nucleus. Axonal branches also recrossed the midline and terminated in identical motoneuron pools on the contralateral side. The above target sites were exactly those expected to be utilized in a reciprocal excitatory-inhibitory fashion during compensatory eye movements. For example, head-down movement would be excitatory for the lower HC producing contractions of both superior recti and inferior obliques as well as relaxation of the antagonistic inferior recti and superior obliques. Therefore, we propose that cells in the anterior vestibular nucleus provide the excitatory pathways to the agonist muscle groups and those in the descending nucleus, the inhibitory connections to antagonists. These data provide a clear example of central rewiring in VOR reflex pathways subsequent to a change in the orientation axes of the semicircular canals and the eye muscle system. Supported by the Grass Foundation, DFG Grant 688/1 and NIH Grant 13742.

269.12 VISUAL-VESTIBULAR INTERACTIONS IN SINGLE UNITS OF RAT VESTIBULAR NUCLEI. K. M. Horn* and S. W. Miller. Department of Psychology, University of Utah, Salt Lake City, Utah 84112.

Single unit electrical activity in the medial and lateral vestibular nuclei of pigmented (Long-Evans) rats was recorded with glass insulated tungsten microelectrodes. Cells were initially located that responded to sinusoidal linear accelerations of a parallel swing (.45 Hz; .13 g) in either the fore-aft or lateral directions in the dark. Responsive units were then tested in the light with vestibular-visual(sinusoidal accelerations of the swing with immobilized visual surroundings), visual (consisting of a large moving visual field with the animal stationary), and conflicting vestibular-visual (linear accelerations with the visual field held constant with respect to the animal) stimulations. An Imsai 80/15 microprocessor was utilized to initiate peripheral stimulation, collect spike frequencies, and generate summary statistics.

The majority of units that were responsive to linear accelerations in a given direction also responded to visual field changes in the direction that simulated the same movement to the animal. For example, if an unit was responsive to movement going towards the posterior of the animal, then the same unit would be responsive to visual stimulation that was proceeding anteriorly. Conflicting sensory input typically led to increased spike frequencies in the response of the units but with attenuated directional sensitivity. Results of this experiment may assist in the understanding of the possible role of visual-vestibular interaction in the generation of motion sickness.

270.1 **A STONE'S THROW AND ITS LAUNCH WINDOW: DID REDUNDANT TIMING CIRCUITS LEAD TO BIGGER BRAINS AND LANGUAGE?** **William H. Calvin, Department of Neurological Surgery, University of Washington, Seattle 98195**

Did bigger brains for more precise throwing lead to human intelligence and language, much as feathers for insulation set the stage for bird flight? Bigger may not be better for intelligence: at least in modern man, size and IQ correlations are poor. There is, however, a bigger-is-faster, faster-is-better relationship to be found in a hominid behavior strongly exposed to natural selection: throwing rocks at prey animals. Faster is better in throwing because it means longer distances and higher kinetic energy for stunning larger animals. Throwing faster (while maintaining accuracy) is not merely a matter of speeding up the "motor tape," as can be seen by simply working backwards from the physics of fairly flat trajectories that bracket a target. Throwing rocks even at stationary prey requires great precision in the timing of rock release from an overarm throw, with releases outside a narrow "launch window" missing the target. But even the launch window for a beginner's throw (about 6 msec for a 6 m throw at a rabbit-sized target) is near the limits of single motoneuron spike timing precision (W. H. Calvin and C. F. Stevens, J. Neurophysiol. 1968), and doubling the target distance to 12 m reduces the launch window to a period about 0.3 msec long at the end of several hundred msec of throwing time.

This suggests that the precision is not achieved at the cellular level. But redundant timers can improve precision, e.g., the 22 chronometers carried on the voyage of the Beagle to reckon longitude. J. T. Enright (Science 1980), modeling circadian oscillator networks, showed that the fluctuation range can be reduced 20-fold by 400-fold redundancy. Thus precision timing can be an emergent property of circuits. Hominids with bigger-than-average brains might have been able to apply more timing neurons to throwing tasks, the success of the faster throws then selecting for encephalization trends (such as neoteny): bigger is faster is better for survival.

Secondary uses of a manual-brachial motor program (rapid uncocking of the elbow) may have included hammering, tool-sharpening, and other features of handedness (W. H. Calvin, Ethology and Sociobiology 1982). Its premotor neural machinery for rapid sequencing may have provided a foundation for language: a peri-Sylvian oral-facial sequencing area (which also has a role in the precision timing of phoneme discrimination) forms the core of modern language cortex (G. Ojemann and C. Mateer, Science 1979). A less desireable consequence of elaborated oscillator and timing circuits may be the temporal lobe's notorious epileptogenicity. (Partial support by NIH grant NS 04053).

270.2 STEROID INFLUENCES UPON ELECTRORECEPTOR TUNING IN WEAKLY ELECTRIC FISH. J. Harlan Meyer and H. H. Zakon. Scripps Institution of Oceanography, Univ. of Calif. San Diego, La Jolla, Ca. 92093.

Weakly electric fish emit electric organ discharges (EODs) for electrolocation and communication purposes. These EODs are detected by electroreceptors, modified hair cells, which are tuned to each animal's own specific discharge frequency. Despite the high stability of discharge frequencies found in these animals, changes do occur: members of the genus Sternopygus show both ontogenetic and seasonal changes in discharge frequencies, resulting in females having higher discharge frequencies than males. This dimorphism could result from steroid influences: androgens cause discharge frequency decreases, while estrogens cause increases.

We investigated the plasticity of electroreceptor tuning in Sternopygus by giving injections of 5α-dihydrotestosterone (DHT) over a 2 week period, while concurrently monitoring the tuning of electroreceptors. Control animals were given saline injections. Two methods were used to assess electroreceptor tuning characteristics. The first involved stimulating the fish with electrical pulses. Such stimuli elicit damped oscillations in electroreceptors, which were recorded extra-cellularly. As the period between cycles of these oscillations is related to the best frequencies of electroreceptors, this method allowed a non-invasive means for repeated determinations of electroreceptor tuning characteristics. The second technique involved determining the best frequencies of primary afferent electroreceptive fibers.

As a consequence of DHT injections, all hormone treated fish showed decreased discharge frequencies. Concomittant with these frequency changes were decreases in electroreceptor best frequencies. The declines in discharge frequencies and receptor best frequencies occurred at similar rates. No significant changes were noted in either discharge frequencies or receptor tuning characteristics in the saline treated control animals.

These results indicate that the tuning characteristics of electroreceptors are dynamic. This plasticity permits changes in an animal's discharge frequency while still allowing the animal to be maximally sensitive to its own discharge.

270.3 NALOXONE-REVERSIBLE SUPPRESSION OF ISOLATION CALL PRODUCTION AFTER MORPHINE INJECTIONS IN SQUIRREL MONKEYS, J. D. Newman, M. R. Murphy* and C. R. Harbaugh*. Lab. of Devel. Neurobiol., NICHD, NIH and Lab. of Brain Evolution and Behav., NIMH, Bethesda, MD 20205.

Many infant mammals respond to maternal separation by the repetitive production of characteristic vocalizations ("isolation calls"). In some species, production of the isolation call persists into adulthood. Previous work has shown that opiates reduce the rate of isolation call production in puppies and guinea pigs (Panksepp, J., et al., Neurosci. Biobehav. Rev., 4:473, 1980). Our study demonstrates that this result may likewise be obtained in the squirrel monkey (Saimiri sciureus). In this species, the isolation call is produced at all ages upon separation from social companions. In our study, subjects 2 or more years old and known to be robust vocalizers when isolated were given parental injections of 10 mg/kg morphine sulfate, then taken immediately to a sound-attenuating chamber. Vocal behavior was monitored over 15 minutes. Typically, a subject was totally silent throughout this time. Examination of subjects at the end of this test showed them to be alert and responsive to external stimulation. Subjects were then given a parental injection of 0.5 mg/kg naloxone or 0.1 ml normal saline, and returned to the isolation chamber for another 15 minutes. Naloxone-injected animals began vocalizing within 4 minutes and continued for variable lengths of time, whereas animals injected with saline were silent throughout this second test period. These data suggest that endogenous opiates may play a role in regulating isolation call production in primates.

270.4 AUTORADIOGRAPHIC PATTERNS OF DEOXYGLUCOSE UPTAKE DURING THE ENTRANCE TO HIBERNATION. T. S. Kilduff, C. P. George*, F. R. Sharp and H. C. Heller*. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305 and Dept. of Neurosciences, University of California, San Diego, CA 92103.

Hibernation is among the most profound examples of global modulation of CNS activity that occurs in the animal kingdom. The entrance process, in which body temperature falls from 37°C to as low as 2°C, occurs over a period of 8-24 hr in the ground squirrel and involves coordinated behavioral and physiological changes. Cortical activity is undetectable by the EEG below a body temperature of 20°C. In order to understand the neural basis of this dynamic process, we have utilized the [^{14}C] 2-deoxyglucose (2DG) technique and compared our results to those we previously obtained during euthermia and deep hibernation (Kilduff et al., 1982, J. Neurosci. 2:143-157). Cold-acclimated golden-mantled ground squirrels, chronically catheterized in the jugular vein, were removed from the colony room during an entrance bout and placed in a metabolic chamber maintained at 5°C. A thermocouple was placed into a subcutaneous re-entrant tube and body temperature and metabolism were continuously measured. The transfer procedure induced a partial arousal which usually reversed at a body temperature of 22-25°C. When body temperature subsequently decreased to 20°C, 2DG was injected (150 uCi/kg) in sterile saline via the catheter and allowed to incubate until temperature to 15°C (2-2.5 hr). The animal was then sacrificed, its brain and spinal cord were removed, frozen, sectioned in a cryostat, and autoradiographed. Approximately 90 neural structures were identified on the autoradiographs and their relative 2DG uptake calculated as the ratio of the optical density of the structure to the optical density of the optic tract. The autoradiographic patterns were highly reproducible between animals. Structures that increased their relative 2DG uptake during entrance compared to both hibernation and euthermia include the lateral hypothalamus, solitary and pontine reticular nuclei. The paratrigeminal and suprachiasmatic nucleus (SCN) increased their relative 2DG uptake during entrance as compared to euthermia. A band of increased uptake extended dorsally from the SCN around the walls of the 3rd ventricle; the relative 2DG uptake of these periventricular nuclei was not measurable due to their narrow width. Since this autoradiographic pattern was seen only during the entrance process and these nuclei are known to contain peptides and other neuromodulatory substances, it is suggested that this pattern may represent neural metabolism associated with release of substances into, or reception of substances from, the cerebrospinal fluid. Such substances may effect the global modulation of CNS activity characteristic of hibernation (Supported by NIH NS10367 to H.C.H.).

270.5 USE OF [^{14}C]-2-DEOXYGLUCOSE TO DETECT REGIONAL BRAIN ACTIVITIES ASSOCIATED WITH FEARFUL, AGGRESSIVE, AND COPULATORY BEHAVIORS IN MALE RODENTS. Robert Blanchard*, Bruce Morton, Milton Diamond*, Eugene Lee* and Clayton Chan*. (SPON: Claire Zomzely-Neurath). Depts. of Psychology, Biochemistry, and Anatomy, Univ. of Hawaii, Honolulu, Hawaii 96822

2-Deoxy-D-glucose (DG) is potentially valuable for identifying brain regions associated with various behaviors. This work employed a densitometric analysis which permitted such associations to be made in males of three rodent species exhibiting defensive, territorial, and sexual behaviors.

Immediately prior to each behavioral period, the experimental and control groups (n=8) received 5 (mice,i.p.) or 10 (hamster, i.p.; rat, jugular cannula) muCi/100g [^{14}C]-DG. These sessions were followed by pentobarbital sacrifice, perfusion via heart-puncture with buffered cacodylate and then fixation with para-formaldehyde. Subsequently the brains were removed, frozen in isopentane-dry ice, sectioned coronally (20 mu) at -18° C, dried on coverslips and placed against Kodak SB5 x-ray film for 3-6 weeks. Two dimensional fiber optic microdensitometry allowed analysis of the resulting negatives. 86 brain loci were chosen for comparison. Computer assistance was used to reveal those regions differing between groups at P 0.05.

Behavioral sessions included unrestrained wild Rattus norvegicus subjected for 45 minutes to various painless stimuli to elicit defensive postures, vocalizations, attacks, and escapes. The frightened experimentals showed 17 brain loci significantly different from unmolested controls including: piriform cortex (-16%), basolateral amygdala (-14%), dorsal central gray (18%), habenula (19%), and pretectum (20%).

Male mice (Swiss-Webster) were singly introduced into a colony male's territory and the resulting interactions observed for 30 minutes. Attacks by the residents and defense by the unwilling intruders were recorded. Comparison of attackers to intruders indicated significant differences in the medial and lateral septum (-22% and -19%), the dorsal central gray (-14%) and the locus coeruleus (19%).

Golden hamster males were exposed to estrus females for 30 minutes and records made of oral-genital contact, mounting, intromission and ejaculatory activities. Males who ejaculated were compared with those who did not mount and males not exposed to females. Most significantly, ejaculating males showed high activity in the globus pallidus (40%) and reduced activities in the lateral thalamus (-41%), posterior cingulate cortex (-33%), dorsal central gray (-31%), parietal and subicular cortex (-27% and -23%).

These preliminary data extend the applicability of DG technique to behaviors as varied as defense, attack, and sexuality.

270.6 RELATIVE SENSITIVITY OF TYMPANIC AND EXTRATYMPANIC SOUND TRANSMISSION IN THE LEOPARD FROG, RANA PIPIENS. Walter Wilczynski, Carl Resler*, and Robert R. Capranica. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y., 14853.

A number of studies (Lombard and Straughan, '74, J. Exp. Biol., 61:71; Pettigrew et al., '78, Nature, 272:138) using multiunit recordings from the midbrain have suggested that in frogs sound can stimulate the inner ear without involving the tympanum. We decided to investigate the relative sensitivities of tympanic and nontympanic sound transmission to the inner ear of the leopard frog (Rana pipiens) by recording the thresholds of eighth nerve auditory fibers at frequencies throughout the frog's range of hearing. The eighth cranial nerve was exposed through the roof of the mouth and a clay cap, molded to mimic the natural mouth cavity, was inserted and sealed with silicon grease, leaving the surgical exposure accessible. The frog was placed on a vibration isolating table. An earphone was sealed around one tympanum to provide a closed sound system, and a speaker was placed on a separate table 0.8 m from the frog to provide free-field stimulation. The contralateral tympanum and external nares were covered with grease to further limit accessibility of the ipsilateral tympanum to free-field stimulation. The thresholds of single eighth nerve fibers to tones presented alternately through the earphone and through the speaker were compared. Below 0.3 kHz, thresholds for the two modes of stimulation were identical. Thresholds remained within 5 dB of each other up to 0.9 kHz. At higher frequencies, threshold separation increased so that at 1.7-2.0 kHz thresholds for tones presented through the speaker were 15-20 dB higher than for tones presented through the earphone. To control for sound inadvertantly entering the earphone housing during free-field stimulation, the tympanum under the earphone was also covered with grease, and the thresholds to tones presented alternately through the earphone and speaker were again determined. After this treatment, thresholds to tones presented through the speaker were lower than thresholds to tones presented through the earphone at all frequencies tested, with the effect most pronounced at low frequencies. These results suggest that the frog's inner ear can be stimulated by both tympanic vibration and by a nontympanic mechanism throughout the frog's range of hearing, and that at low frequencies both modes of stimulation have nearly equal sensitivity. We are presently investigating how the interaction of these two acoustic inputs could provide potential sound localization cues.

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270.7 PROCESSING OF AMPLITUDE MODULATED SIGNALS BY THE PERIPHERAL AND CENTRAL AUDITORY SYSTEMS OF THE LEOPARD FROG. G. J. Rose and R. R. Capranica. Section of Neurobiology and Behavior, Langmuir Lab., Cornell University, Ithaca, NY 14853.

Amplitude modulation is a predominant temporal feature in the vocalizations of the leopard frog, Rana pipiens. The neural mechanism of processing this aspect of sound was investigated by recording single unit activity in the torus semicircularis and in the eighth cranial nerve. Acoustic stimuli consisted of pure tones and sinusoidally amplitude modulated white noise. The sound pressure level of the stimulus was held constant while the rate of amplitude modulation was varied over the range 8 Hz to 150 Hz. At each value of amplitude modulation (AM) tested, the spike-rate and the degree of phase-locking of the response to the stimulus waveform (synchronization) was computed. The spike-rate of eighth nerve fibers was largely independent of the rate of AM. For the majority of these units, the degree of synchronization remains relatively constant up to approximately 100 Hz AM and significant synchronization often was observed up to 150 Hz AM. Broadly tuned fibers with best excitatory frequencies greater than 1200 Hz (presumably innervating the basilar papilla) were an exception to this pattern. The synchronization of these units improved as the rate of AM was increased from 8 Hz to 100 Hz. In contrast, the spike-rate of most toral units was a function of the rate of AM. In addition, a wider range of synchronization values was found among the toral cells relative to eighth nerve fibers. The selectivity of toral neurons, as measured from the response level at each rate of AM, was not evident in the synchronization analysis. Rather, most toral neurons exhibited uniform synchronization at the lower rates of AM and progressively weaker synchronization at the higher rates of AM. (Supported by NINCDS grant NS-09244).

270.8 EM ANALYSIS OF PRIMARY ELECTRORECEPTIVE AFFERENTS, LABELLED BY INTRACELLULAR INJECTION OF HRP. W.Heiligenberg and L.Maler . Scripps Institution of Oceanography, Univ. of Calif. at San Diego, La Jolla, CA 92093 and Dept. of Anatomy, Fac.Med., Univ. of Ottawa, Ontario K1N 9A9, Canada.

Tuberous electroreceptors are driven by the animal's own periodic electric organ discharges (EODs). Feedback from these discharges is commonly modulated due to the presence of objects or interfering EODs of conspecifics, and two kinds of modulations are of particular relevance to the animal: modulations in instantaneous phase and modulations in instantaneous amplitude. Two classes of tuberous electroreceptors, T- and P-units (Scheich et al 1973, J.Neurophys. 36,39-60) are known which predominantly, but not exclusively, encode modulations in phase and amplitude respectively. Primary afferents from these two types of receptors terminate in 3 somatotopically ordered areas of the posterior lateral line lobe (PLLL, Heiligenberg and Dye 1981, Neurosciences Abstr. 272.5), a multilayered structure of the hind brain. Within the PLLL, cell types are found which encode either modulations in phase or modulations in amplitude in a more exclusive manner than one observes at the level of the two types of primary afferents. This further specialization in coding obviously results from the neuronal wiring of the PLLL and the nature of synaptic contacts of T- and P-afferents. In order to determine the organization of synaptic contacts, terminations of physiologically identified, intracellularly HRP-labelled afferents were studied electronmicroscopically.

Our results largely support the original contention of Maler et al (1981, J.Comp.Neurol. 195,87-193) that T-afferents terminate on spherical cells and P-afferents terminate on granule, basilar pyramidal and polymorphic cells. We have, however, found exceptions to this rule, such as terminations of one T-afferent on spherical and granule cells. It appears now that Maler's original rule only holds in a statistical and quantitative rather than strictly qualitative sense. This conforms with the observation that T and P-type afferents represent ends of a spectrum of tuberous afferents rather than strictly separate classes.

270.9 IDENTIFIED PHASE CODER NEURONS IN THE ELECTRIC FISH TORUS
C. Carr and W. Heiligenberg.UCSD, A-002, La Jolla, CA, 92093.

The Gymnotiform fish Eigenmannia produces a high frequency electric organ discharge (EOD) which it uses for electrolocation and social interactions. These fish possess a jamming avoidance response (JAR) whereby they are able to raise or lower their EOD frequency so as to avoid interference from a neighbouring fish of a similar frequency. The JAR requires that the fish be able to evaluate the simultaneous changes in amplitude and phase which occur when its EOD is contaminated with that of a neighbour's, and then that it produce an appropriate shift in its EOD frequency.

Eigenmannia evaluates these changes in phase and amplitude information via an array of electroreceptors distributed over the body surface. Receptors are innervated by phase coder (T-type) or amplitude coder (P-type) primary afferents which project somatotopically to different laminae of the ipsilateral posterior lateral line lobe (PLLL). PLLL T- and P-units project somatotopically to different laminae of the midbrain torus semicircularis (TS).T-units project to lamina 6, P-units to laminae 9,8d,8b,7,5 & 3. The fish must combine this phase and amplitude information in order to perform the computations necessary for the JAR. This can only occur at the level of the TS, as phase and amplitude information are segregated up to this level, and phase information is confined to lamina 6 which has no efferent projections. We are looking for the site of such phase and amplitude comparisons.

Intracellular recording and marking with HRP or Lucifer Yellow has enabled us to identify a single class of giant (40μ) T-type cell in lamina 6. Each giant cell receives an input from 3-4 contralateral PLLL T-units. Intracellular labelling of these PLLL units demonstrates that each forms a dense basket of terminals around the soma of a single giant cell.The morphology of the giant cells is dependent upon which part of the body they receive their input from, due to the somatotopic organization of the TS. All giant cells have two or more thick(5μ) axons, at least one of which is contralateral, making a homotypic connection to the vicinity of its contralateral homologue, thus connecting equal but opposite sides of the body. Giant cells in the more caudal TS which receive their input from trunk T-units generally send another contralateral axon to a more rostral region of lamina 6. Rostrally situated giant cells send a second axon to the more caudal contralateral TS. All giant cells have at least one ipsilateral axon which is either rostrally or caudally directed dependent upon the cell's location. A large dendritic tree surrounds the soma. These intrinsic connections in lamina 6 provide a substrate for the phase comparisons essential for the performance of the JAR. Intracellular recordings from these cells, however, suggest that they do not perform these comparisons themselves, but rather project to a second class of phase comparator cell.

270.10 SPECIALIZATIONS IN NUCLEUS AMBIGUUS OF THE BAT PTERONOTUS PARNELLII: RELATION TO FINE FREQUENCY CONTROL OF BIOSONAR VOCALIZATIONS.
J.B. Kobler*, J.H. Casseday and O.W. Henson, Jr.* Neurobiol. Prog., Univ. of N. Carolina, Chapel Hill, N.C., 27514, Depts. of Surgery (Otolaryngology) and Psychol., Duke Univ., Durham, N.C., 27710, and Dept. of Anat., Univ. of N. Carolina.

By controlling the frequency of its brief ultrasonic biosonar pulses the bat Pteronotus parnellii cancels shifts in echo frequencies due to moving targets or changes in its own flight speed (Doppler-shift compensation). The bat thus maintains echo frequencies within a narrow band to which it is especially sensitive. These observations suggest that Pteronotus has extremely fine laryngeal control and that this control is closely related to sensory input. To begin a study of the neural pathways comprising this auditory-laryngeal feedback system, we have identified the brainstem projections to the larynx, with particular attention to the cricothyroid muscle (CTM), known to have an important role in the control of vocalization frequency. Horseradish peroxidase (HRP) was applied separately to the superior laryngeal nerve and its subdivisions which innervate different parts of the CTM as well as to the recurrent laryngeal nerve, the pharyngeal plexus and the descending vagal trunk.

The results identified the nucleus ambiguus (AMB) as the origin of neurons innervating the larynx, as in other species. However, compared to other mammals, there are two major differences in the projections and organization of AMB: (1) The CTM is innervated by many more neurons (3000-4000) than has been reported in other species. We also compared counts of HRP-labeled cells or counts of axons in nerve sections with muscle volumes measured by computer-aided 3-D reconstructions. There are about six times more efferent axons per mm^3 of muscle in the CTM than in the other intrinsic laryngeal muscles. Small motor units and high packing density of muscle fibers in the CTM may thus be the basis of fine frequency control. (2) Within the AMB two distinct cytoarchitectural divisions innervate the CTM. Differences in the origins of the motor pathways appear to correspond to structural differences between the anterior and posterior parts of the CTM. The anterior ventral division of AMB, not described in other species, innervates the anterior CTM. The anterior division of AMB innervates primarily the posterior CTM.

The remaining projections of AMB are as follows: The posterior dorsal division projects to the other intrinsic laryngeal muscles; the dorsolateral division projects to the pharyngeal muscles; vagal preganglionic cell bodies are found in anterior dorsomedial and posterior ventral areas.

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270.11 STEREOPSIS AS A PRIMARY CUE FOR DEPTH PERCEPTION IN THE PIGEON.
Sally McFadden* and J. Martin Wild (SPON: D.J. TRACEY). Dept. of Behavioural Biology, RSBS, Australian National University, Canberra ACT 2600 Australia.

Some degree of binocular overlap is regarded as necessary for stereoscopic depth perception, in which the relative separation of objects in depth is determined by the disparity between the retinal images of the two eyes.

Despite the fact that nearly all vertebrates have a binocular field, stereopsis has only been demonstrated behaviourally in a few species - man, cat, monkey and falcon - all of which have frontally placed eyes. In contrast, the pigeon's eyes are laterally placed, giving a panoramic visual field with only a small region of binocular overlap. The present study indicates that this overlap enables good stereoacuity.

Pigeons were trained in a discrete trial, simultaneous discrimination paradigm in which back-illuminated stimuli were presented directly behind the pecking keys. The stimuli were adapted from J. Frisby (see Hinchcliffe, H., Brit. Orthopt.J., 35: 46-57, 1978). One consisted of a random pattern of triangles on the front of a clear piece of glass. The other stimulus had a center, circular portion of the pattern on the back of the glass, giving the impression of a circle displaced in depth. When viewed monocularly without motion parallax, the circle disappeared and the pattern appeared in one plane. Depth thresholds could be systematically obtained simply by varying the thickness of the glass.

Subjects were trained to a criterion of 90% correct under binocular conditions. One eye was then occluded resulting in a reduction of stimulus control to chance levels. Monocular performance never reached binocular criterion levels even after extended training. Estimation of the magnitude of the retinal disparity corresponding to the separation in depth of the stimuli at threshold was less than 12 min of arc, a value within the range of disparities best detected by the falcon (Fox, R., et al., Science 197: 79-81, 1977).

These behavioural results complement those from anatomical (Miceli, D., et al., Brain Res. 100: 125-131, 1975) and physiological (Perisić, M., et al., Int.J.Neurosci. 2: 7-14, 1971) studies which demonstrate a bilateral projection from the thalamus to the visual wulst in the pigeon and the presence there of cells which can be driven from both eyes. A neural pathway possibly subserving stereopsis thus exists in the pigeon.

270.12 LIGHT-ADAPTED SPECTRAL SENSITIVITY IN HAPLOCHROMIS BURTONI (CICHLIDAE).
E. E. Allen*& R. D. Fernald (SPON: M. Gordon-Lickey). Biol. Dept., Univ. of Ore., Eugene, Ore. 97403.

Many of the social interactions of the African cichlid H. burtoni are mediated by vision. Color patterns of adult territorial males as well as spectral "windows" in the turbid waters in which these animals live may be matched to photoreceptor pigments. The retina contains rods with a vitamin A_1 based pigment absorbing maximally at 500 nm. The pigments $P455_1$, $P523_1$, and $P562_1$ occur individually in cones which are arrayed in a rectangular mosaic which minimizes spacing between different receptor classes (Fernald , R. D. & Liebman, P. A., Vis. Res. 20: 857-864, 1980).

Experiments are underway to determine if this retinal pigment organization corresponds to functional trichromatism. A two-choice, food reward, operant conditioning paradigm is being used to assay spectral spensitivity. The dark adapted luminous efficiency function (LEF) has been found to correspond closely to the nomogram for a $P500_1$ pigment (Allen, E. E. & Fernald, R. D., Neurosci. Abs. 7: 270, 1981). The light adapted LEF is broader and less sharply peaked, indicating that it is based on interactions among several pigments. Maximum sensitivity is found in the mid-spectral region with the decline being most pronounced at the long wavelength end. These findings are consistent with photopic visual processes being mediated through an opponent processing system similar to that found in known trichromats.

Wavelength discrimination and purity discrimination tests will be carried out to determine unambiguously the presence or absence of trichromatism.

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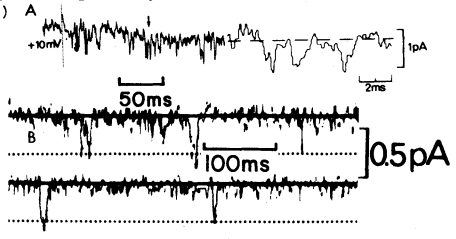
271.1 PHYLOGENY OF SINGLE CHANNEL CALCIUM CURRENT IN NEURONS. H. D. Lux*, D. L. Kunze, H. Camerer* and A. M. Brown*. University of Texas Medical Branch, Galveston, TX and Max-Planck-Institut fur Psychiatrie, Munich, FRG.

Voltage-dependent membrane Ca currents trigger such basic and diverse neuronal functions as spontaneous activity and the coupling of excitation with secretion. These functions probably arose early in neuronal evolution and we wondered if unitary Ca currents were similar among neurons from different species having these functions. We studied spontaneously active neurons from Helix, as examples of pacemaker and molluscan neurons, the PC12 line of cells, as examples of neurosecretory and vertebrate mammalian neurons and chick dorsal root ganglion neurons (DRG) as examples of vertebrate, non-mammalian neurons. The gigaseal method was used for detecting unitary currents and in the case of the snail neurons, a double microelectrode voltage clamp was used simultaneously for whole cell clamping. Cell-attached, and inside-out patches were examined and whole cell clamping on PC12 and DRG neurons was also done. Ca currents were isolated by suppressing Na currents with TTX and Tris substitution and by suppressing K currents with Cs substitution, TEA and 4-AP. The anions were Cl^- or gluconate and pH was adjusted to 7.4 for extracellular solutions and 7.2 for intracellular solutions. The inset shows unitary Ba currents in a cell-attached DRG patch and unitary Ca currents in a cell-attached snail neuron. Unitary currents are small, brief and clustered. Data were collected and analyzed from over 200 patches on snail neurons, 13 patches from DRG neurons and 16 patches from PC12 neuron. Amplitudes were similar in all cells and were 0.5 pA at -10 to +10 mV. Average open times were similar for snail and PC12 cells and were about 2.7 msec; they were shorter for DRG cells. Unitary activity was clustered and two distributions of closed times were observed with average values of about 1.6 and 35 msec. Based on the single channel results, we conclude that unitary Ca currents are similar in neurons having different functions in widely divergent species. (Supported by NIH grants NS-11453, HL-25145 and NSF grant PCM-78232)



271.2 FAST AND SLOW COMPONENTS OF CALCIUM INACTIVATION IN APLYSIA NEURONS EACH EXHIBIT CURRENT DEPENDENCE. R. Eckert and D. Ewald*. Department of Biology and Ahmanson Laboratory of Neurobiology, UCLA, Los Angeles, CA 90024.

The calcium current, I_{Ca}, recorded under voltage clamp depolarization in molluscan neurons partially inactivates to a maintained current level along a time course consisting of a fast exponential phase, τ_{h1}, and a slower exponential phase, τ_{h2}. The fast component of inactivation is apparent only in Ca currents of medium to large amplitude, disappearing if for any reason (e.g., small depolarization, inactivation due to prior Ca entry, low Ca concentration, or partial current block by Cd^{2+}) the peak I_{Ca} amplitude is small (<0.1 μA in Aplysia neurons). During brief depolarizations with moderate to strong Ca entry, inactivation is nearly fully dominated by τ_{h1}, which was shown to depend on Ca^{2+} entry and accumulation (Eckert and Tillotson, J. Physiol. 314: 265-280). We provide evidence now that τ_{h2}, like τ_{h1}, is primarily current dependent in Aplysia neurons. I_{Na} and I_K were blocked with 0.45 mM TTX, 200 mM TEA, and 5 mM 4-AP in ASW containing 20 mM Ca^{2+}, and I_{Ca} was recorded during 900ms depolarizations of axotomized neurons L2-6 clamped to potentials of -30 to 0 mV from a -40mV holding potential. Under normal conditions, the time constant of the slow component, τ_{h2}, is about 300 ms at 0 mV. The time constant was longer if the depolarization (and hence I_{Ca}) was smaller; thus at -25 mV τ_{h2} was about 700 ms. A similar slowing in the kinetics of τ_{h2} took place at a given pulse voltage (i.e., 0 mV) when I_{Ca} was reduced by partial block of the Ca channels with 0.5 mM Cd^{2+}. Thus, τ_{h2} is current dependent. However, the kinetics of τ_{h2} for a given current amplitude showed some voltage sensitivity, i.e., τ_{h2} was slower for stronger depolarizations. The degree of inactivation attained at any time along the τ_{h2} trajectory during the depolarization had a nearly linear relation to the time integral of I_{Ca} (i.e., prior Ca entry). The degree of inactivation relative to the I_{Ca} time integral was a function of membrane voltage, becoming smaller with increasing depolarization. This effect may be related to the voltage-sensitivity of the Ca-mediated inactivation of the Ca conductance (Eckert and Ewald, 1981, Biophys. J. 37:182a). Finally, injection of EGTA effects a slowing of τ_{h2} as well as τ_{h1}, providing further evidence that both fast and slow components of inactivation are mediated by entry and accumulation of Ca^{2+}. We propose that τ_{h1} and τ_{h2} represent two rates at which Ca ions entering the cell during depolarization become unavailable to receptor molecules mediating the inactivation of the Ca channels. Supported by USPHS NS 8364 and NSF BNS 80-12346.

271.3 CALCIUM CHANNEL INACTIVATION IN THE PRESENCE OF DANTROLENE SODIUM IN FROG TWITCH MUSCLE FIBERS. G. Cota* and E. Stefani. Department of Physiology and Biophysics, Centro de Investigación y de Estudios Avanzados del I.P.N., Apartado Postal 14-740, México, D.F. 07000, México.

Ca entry is not indispensable for inactivation of the calcium channel in intact frog twitch muscle fibers under hypertonic sucrose solution. Evidence for this view is given for the following observations: 1) In two pulse inactivation experiments, Ca current (I_{Ca}) or barium current (I_{Ba}) during the test pulse can be reduced to about 50% without any detectable inward current during the prepulse (7 sec); 2) I_{Ca} or I_{Ba} rate of decay is not related to the peak current amplitude; 3) The rate of decay measured at comparable membrane potentials is the same for both I_{Ca} or I_{Ba} (Cota, G., Nicola Siri, L. and Stefani, E., Biophys. J., 37:316a, 1982). However, these results do not rule out an intracellular Ca-dependent inactivation mechanism since Ca is released from the sarcoplasmic reticulum (SR). Therefore, we tested the effect of dantrolene sodium on I_{Ca} inactivation. It is thought that dantrolene may inhibit internal Ca release. Experiments were performed on cutaneous pectoris muscle fibers from Rana moctezuma. Initially, we studied the effect of dantrolene on Ca release from the SR. To this end, we analyzed the kinetics of mechanical activation. The recording solution was (mM): TEA-CH_3SO_3 120 and $Ca(CH_3SO_3)_2$ 10. Mechanical threshold at 18-19°C was optically detected by using the two microelectrode voltage clamp technique. The addition of 10-15 μM-dantrolene to the recording solution increased mechanical threshold. This effect was more pronounced for short pulses. For example, the mechanical threshold for 50 and 1 msec pulses was respectively -54±1 mV (7) and +14±2 mV (6) in control solution, and -50±1 mV (6) and +40±4 mV (5) with dantrolene added. This observation is consistent with the idea that dantrolene reduces Ca release from SR upon depolarization. To record I_{Ca}, the three microelectrode voltage clamp method was used and 350 mM sucrose was added to the recording solution to abolish contraction. Outward currents were blocked by preincubating the muscles overnight at 4°C with continuous agitation in (mM): TEA-Cl 60, CsCl 60 and $CaCl_2$ 1.8. Two pulse inactivation experiments were performed at 22-23°C. The I-V curve, the rate of decay and the inactivation curve for I_{Ca} were not significantly modified by 10-15 μM-dantrolene. Inactivation curves were fitted to $h_\infty=\{1+\exp|(E_m-\bar{E})/k|\}^{-1}$ with $\bar{E}$=-44±1 mV and k=9.1±0.4 mV (6) in control solution and $\bar{E}$=-46±1 mV and k=8.4±0.2 mV (7) with dantrolene added. Thus, it appears that neither Ca entry nor an internal Ca normal release are indispensable for the inactivation of the calcium channel, suggesting that this process may be under direct control of the voltage membrane.

271.4 EVALUATION OF ARSENAZO III-Ca^{2+} STOICHIOMETRY IN THE PRESENCE AND ABSENCE OF ETHYLENE GLYCOL-BIS-(2-AMINOETHYLETHER)-N,N'-TETRA-ACETIC ACID (EGTA) AT DIFFERENT pH. U. Pande* and H. C. Pant. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Arsenazo III has been used as an indicator for the measurement of micromolar amounts of free Ca^{2+}, but its binding stoichiometry with Ca^{2+} is still not clear. We have analyzed the Arsenazo-Ca^{2+} binding using low concentrations of Arsenazo (9 μM) at various pH's and found that: (a) change in the absorbance of the Arsenazo-Ca^{2+} complex at 650 nm with Ca^{2+} depends upon pH even in a narrow range from pH 6.55 to pH 7.32; (b) at a fixed Ca^{2+} concentration the absorbance at 650 nm decreases with increasing pH in the presence of EGTA but increases with increasing pH in the absence of EGTA (these results are similar to those found by Brown and Rydquist, Biophys. J., 36: 117, 1981); (c) at a constant pH and Ca^{2+} concentration, the increase in absorbance of the Arsenazo-Ca^{2+} complex with an increasing Arsenazo concentration is different in EGTA buffered and EGTA unbuffered systems. These results are analyzed by assuming nCa^{2+} ions bind with one Arsenazo (Az) molecule in equilibrium according to the reaction

$$nCa + Az \underset{K_D}{\rightleftharpoons} Ca_n\,Az \qquad (\text{where } \frac{1}{K_D} = k_{b1}\,k_{b2}\text{---}\,k_{bn})$$

which consists of a sequence of binding steps each with its own binding constant k_b. Rearranging the equilibrium equation gives:

$$\ln [Ca_n\,Az] = n\ln [Ca] + \ln [Az] + \ln \frac{1}{K_D}$$

In pH range 6.55 to 7.3, plots between ln $[Ca_n\,Az]$ and ln [Ca] in EGTA unbuffered systems give a slope of one (n=1), i.e. the binding stoichiometry is 1:1. In EGTA buffered systems the slope gradually changes from one (stoichiometry 1:1) at pH 6.55 to two (stoichiometry 2:1) at pH 7.26. An estimate of the binding constant k_{b2} of CaAz (1:1) complex with Ca is also made in EGTA buffered systems by calculating K_D from the intercept of plots and taking k_{b1} value from the literature as $10^5 M^{-1}$. It appears that with increasing pH from 6.55, k_{b2} increases from $10^2 M^{-1}$ and becomes comparable to k_{b1} at pH ≥ 7.25, facilitating the formation of a 2:1 complex at pH ≥ 7.25. It is suggested that in EGTA buffered systems at higher pH, the CaEGTA complex interacts with Arsenazo, thus yielding a 2:1 stoichiometry. Since Arsenazo III exists in solution in multiple states of protonation, this model fits with the structural changes that might occur in Arsenazo III with pH. Using the above analysis, calcium binding with erythrocyte ghost membranes is discussed.

271.5 THE CALCIUM CURRENT OF ROD-PHOTORECEPTOR INNER SEGMENTS RECORDED WITH A WHOLE-CELL PATCH CLAMP. D. P. Corey, J. Dubinsky and E. A. Schwartz*. Dept. of Pharm. & Physiol. Sci., Univ. of Chicago, Chicago, IL and Dept. of Physiol., Yale Med. Sch., New Haven, CT.

The voltage-dependent calcium current of photoreceptors is of particular interest in understanding the processing of visual information, as it serves both to modify the receptor potential and to mediate the continuous, Ca^{++}-dependent release of transmitter. We have studied this current in dissociated rod inner segments from the retinae of tiger salamanders (Ambystoma tigrinum), with a whole-cell patch-clamp technique. Single micropipettes of ~0.5 um tip diameter were sealed onto inner segments by mild suction; strong suction was then applied to rupture the membrane under the electrode to provide low resistance access to the cell interior. With compensation for series resistance, the voltage clamp settled in 300 usec. The cell input resistance was initially 2.1±1.3 GΩ at -70 mV, and capacitance was 12.5±3.6 pF.

The Ca^{++} current was isolated by replacing the bath solution with 100-mM TEA Methanesulfonate + 6-mM Ca^{++} and by allowing the cytoplasm to exchange with TEA Aspartate + 10-mM EGTA in the pipette. Under these conditions, input resistance at -70 mV increased to 15±10 GΩ. At -30 mV an inward current appeared; it peaked at -5 mV and approached zero above +50 mV. The peak inward current ranged from 10 to 100 pA at the start of a recording, and declined to near zero during the following 10-20 min. This current was identified as a Ca^{++} current, because it increased slightly when Ca^{++} was replaced with Ba^{++}, was insensitive to TTX and STX, and was blocked by D-600 or replacement of Ca^{++} with Co^{++}.

At 10° C the onset of current following a depolarizing voltage step had a sigmoidal shape; the time constant of the rate-limiting step decreased from 10 msec at -20 mV to 2 msec at +25 mV. Tail currents observed following a hyperpolarizing step could be fitted with a sum of two exponential components; the dominant, fast component had a time constant ~0.8 msec at -20 mV.

With 10-mM EGTA in the pipette solution, we saw no inactivation of the current, even with 3-sec voltage steps to 0 mV. When EGTA concentration was decreased to 0.1 mM, there was a decline in the current with time. Inactivation thus appears to be Ca^{++}-dependent but not voltage-dependent. Estimates of the change in intracellular Ca^{++} concentration required to produce inactivation suggest that Ca^{++}-dependent inactivation does not normally occur under physiological conditions.

(Supported by NIH grants EY-02440 to EAS and NS-12961 to C. F. Stevens.)

271.6 **LIGHT-INDUCED CHANGES OF INTRACELLULAR Ca^{2+} IN HERMISSENDA PHOTORECEPTORS MEASURED WITH ARSENAZO III.**

J. Connor, Bell Labs, Murray Hill, NJ, 07974 and D. L. Alkon, Lab. of Biophysics, NINCDS, NIH, MBL, Woods Hole, MA 02543.

Type B photoreceptors in the eye of Hermissanda respond during a light step with a biphasic depolarization. Following a light step of moderate to bright intensity ≥ $10^{3.5}$ ergs/cm^2-sec) the Type B cell remains depolarized for 1-2 minutes. The magnitude and duration of this long-lasting depolarization (LLD) is altered by a conditioning paradigm (but not control procedures) in which light is repeatedly paired with rotation (2.0 g maximum follows light onset by ~1.0 s). Previous work has indicated that a light-induced, voltage-dependent Ca^{2+} current contributes importantly to the generation of the LLD (Alkon, Science 205, 810, 1979). To further clarify the involvement of Ca^{2+} in producing the LLD, experiments were run using the indicator dye, arsenazo III (Arz). Cells were impaled with a single microelectrode containing 10 mM Arz (R>100 megohms) and filled by iontophoresis (0.5-1.0 nA negative current for 10-15 min). Voltage responses of the cell to light were monitored simultaneously with transcellular absorbance, measured by a fiber optic detection system. The collecting fiber was 40 μm in diameter, slightly larger than the minor diameter (25-30 μm) of the photoreceptor somata, where absorbance was measured. The light response was not degraded by the presence of Arz. Dark adapted (~10 min) cells responded to a 4-7 sec light stimulus (≥ 10^4 ergs/cm^2-sec) with a biphasic depolarization of 20-30 mV peak amplitude followed by a LLD lasting 1-2 minutes. Optical measurements were not possible during the stimulus, but post-stimulus absorbance (ΔA), measured differentially at the wavelength pair 660-690 nm, was markedly greater than the baseline and continued to increase for 15-20 sec, indicating an increase in cytoplasmic Ca^{2+}. ΔA decreased back toward baseline with a time course of 1-2 min, which roughly paralleled the LLD time course. Steady hyperpolarizing current greatly reduced both the duration of the LLD and the ΔA. No ΔA was observed in uninjected cells. The ΔA of injected cells did not reflect Ca^{2+} entry due to impulse activity since similar response occurred both in intact cells and those severed form the impulse initiating zone of the axon. These findings suggest that elevation of cytoplasmic Ca^{2+} during the LLD is voltage-dependent and thus should also be enhanced when light is paired with rotation during the conditioning procedure.

271.7 VOLTAGE-DEPENDENT GAP JUNCTIONAL CONDUCTANCE BETWEEN FISH EMBRYONIC CELLS. R.L. White*, D.C. Spray, A. Carvalho*, and M.V.L. Bennett. Dept. Neuroscience, Einstein Coll. Med., Bronx, N.Y. 10461.

Conductance of gap junctions between amphibian embryonic cells is steeply dependent on transjunctional voltages of either polarity (Spray et al., J.Gen.Physiol. 77:95,'81), and the kinetics of the conductance change are first order (Harris et al., ibid.). We now report that the junctional conductance (g_j) between embryonic cells of the killifish, Fundulus, is also voltage dependent, although to a lesser degree and with somewhat more complex kinetics than in amphibia. Fundulus eggs were fertilized, allowed to reach the 32-64 cell stage, treated with colchicine to inhibit mitosis, and dissected as single cells which were reassociated as cell pairs. Each cell of the pair was voltage clamped with an independent voltage clamp circuit to the same transmembrane potential. Command pulses were then delivered to one cell of the pair, and current (corresponding to g_j) was measured in the other cell. Junctional current initially increased sharply to a value corresponding to the resting conductance, then decayed to a new steady-state value which depended on the command voltage. Plots of steady state g_j as a function of transjunctional voltage are well fit by a Boltzmann distribution:

$$g_j=(g_{max}-g_{min})/(1+\exp(A(V-V_o)))+g_{min}$$

where g_{max} and g_{min} are the maximum and minimum value of g_j, g_{min} =0.2 g_{max}, A=0.06 and V_o=27.1 mV. These values are different from those measured in amphibia (A=0.25 and V_o=14.1 mV; g_{min}=0.05 g_{max} Spray et al., ibid) and may reflect a lower energy difference between the open and closed states of the gap junctional channel in Fundulus as compared with amphibia. Analysis of the decay of transjunctional current from peak to steady state values also shows differences between Fundulus and amphibians. Whereas this relaxation is a single exponential in amphibians, two exponentials are required to fit the Fundulus data over much of the voltage range. Both time constants are voltage dependent; the faster time constant is maximal at higher transjunctional voltages (τ=200 ms at 100 mV), and the slower time constant is most easily seen at intermediate voltages (τ=1100 ms at 50 mV). These data show that fish as well as amphibia possess voltage dependent gap junctions, although with somewhat different time and voltage dependencies. Voltage dependence of junctional conductance may be a widespread property of vertebrate embryonic cells.

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271.8 PHARMACOLOGICAL ALTERATION OF GATING PROPERTIES OF THE GAP JUNCTION CHANNEL. A. Carvalho*, D.C. Spray, R.L. White*, and M.V.L. Bennett. (SPON: L.M. Hall). Albert Einstein Col. Med., Bronx,NY 10461.

Gap junctional conductance (g_j) between pairs of blastomeres from embryos of fish or amphibia is sensitive to transjunctional voltage (Spray et al., J.Gen.Physiol. 77:95,'81; White et al., Neurosci. Abstr., '82) and cytoplasmic pH (pH_i, Spray et al., Science 211:712,'81). Both physiological and pharmacological evidence suggest that voltage and pH_i act upon separate "gates" at the channel level: when g_j is reduced at low pH_i, the residual conductance exhibits the same voltage dependence as at untreated junctions, and specific aldehydes selectively reduce sensitivity to voltage or pH_i (Spray et al., Biophys J. 33:108a, '81). We now present further evidence that the gates are pharmacologically distinct, based on effects of drugs that reduce pH_i dependence without affecting either voltage dependence or g_j itself. Experiments were conducted on pairs of blastomeres from Rana pipiens or Fundulus heteroclitus. The drugs used were N-ethoxycarbonyl-2-ethoxy-1,2-di-hydroquinoline (EEDQ) and retinoic acid (VIT.A). Stock solutions of both drugs were prepared in DMSO and were freshly diluted during the experiments to final concentrations ranging from 200 to 800 μM for EEDQ and 10 to 100 μM for VIT.A. When directly applied to the blastomeres in these concentrations, both drugs had no effect on coupling, although higher doses of VIT.A did decrease g_j. Bath applied weak acids (lactate and acetate substituted for Cl or equilibration of the normal solution with 100% CO_2) acidified the cell cytoplasm and decreased g_j. Addition of either VIT.A or EEDQ along with a weak acid depressed the pH_i dependence of g_j or reversed the effect of the previously applied weak acid. The voltage dependence was unaffected by either EEDQ or VIT.A. Experiments in which pH_i was monitored with a Thomas-type pH microelectrode exclude the possibility of drug action through a simple increase or stabilization of pH_i; administration of these drugs generally slightly decreased pH_i. The experiments demonstrate that EEDQ and VIT.A render the channels less sensitive to pH_i variations without reducing total junctional conductance or altering the voltage gate. The results provide further support for the concept of two distinct gates acting on the gap junction channel.

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271.9 PHOTOLABILE PROTON DONORS AND pH CONTROL OF GAP JUNCTIONS. Jeanne M. Nerbonne, Henry A. Lester and John A. Connor*. Division of Biology 156-29, California Institute of Technology, Pasadena, CA. 91125, *Bell Laboratories, Murray Hill, New Jersey 07974.

We have synthesized three classes of photosensitive molecules capable of liberating protons efficiently upon irradiation (quantum yields 0.25-0.50). Various structural modifications have been incorporated to optimize the photochemical properties (absorption maxima, conversion efficiencies, etc.), thermal stabilities, water solubilities and the general biological usefulness of these compounds. The photoactivatable molecules were designed to be exploited as physiological probes of intracellular pH buffering capacities and to be utilized to study the role of pH in the modulation or regulation of specific ion channels. The first class of proton donors, ortho-nitrobenzylacetates, have been studied in the salivary gland of the midge, Chironomus. The cells in the gland are electrically coupled via gap junctions and the electrophysiological properties of these junctions have been studied extensively by others. Presently available evidence, however, does not allow for a clear decision on the relative roles of intracellular protons versus calcium ions in controlling gap junction permeability. When whole glands are bathed in the parent compound (this molecule is lipid soluble and, therefore, equilibrates in the cytoplasm rapidly) and irradiated, there is a precipitous change in junctional resistance, consistent with a drop in intracellular pH. Similar results are obtained with cell pairs, isolated by mechanical dissociation of the gland. Neither the unphotolyzed compound, the nitroso photoproduct, nor light alone produce uncoupling. Changes in extracellular pH, similarly, have no effect on coupling ratios. Uncoupling begins within 1 sec, although complete uncoupling requires 2-3 min following a single light flash. The dose-dependence of this time course is highly variable although it is possible to conclude that large pH jumps (500 μM-1 mM H^+) produce uncoupling 3-4 times faster than smaller jumps (<250 μM). In vitro studies reveal that the light induces a pH drop in less than 300 msec; preliminary data show that this change is followed by a much slower (half time = 1 min) phase of proton release in some derivatives in the ortho-nitrobenzylacetate series. The absolute time course of the light induced pH-jumps, the role of molecular structure, as well as the relationship between the rate of physiological uncoupling and chemical structure will be discussed.

271.10 BIDIRECTIONAL TRANSMISSION INDUCED AT A RECTIFYING ELECTROTONIC SYNAPSE BY REVERSING JUNCTIONAL POLARIZATION. C. Giaume* and H. Korn, INSERM U3, CHU Pitié-Salpêtrière, 75013 Paris, France.

Synaptic transmission at the Giant Motor Synapse (GMS) is classically described as being electrotonic and rectifying as, at rest depolarizations including action potentials are transmitted from the Lateral Giant Axon (LGA) to the Giant Motor Fiber (GMF), while only hyperpolarizations spread in the opposite direction (Furshpan E.J. and Potter D.D., J. Physiol., 145: 289-325, 1959). Because we found a so far unreported difference (ΔV) between the membrane potential of the presynaptic cell (-90 mV) and that of the postsynaptic one (-75 mV) we were led to investigate possible correlations between this junctional polarization and rectification. Hence coupling was studied by inserting two microelectrodes on each side of the GMS (one for current injections, the second for voltage recording). More specifically, a prolonged polarizing pulse (about 500 msec long) was applied to one element in order to modify ΔV and a second, brief one, was injected at the other side during this period, in order to calculate coupling parameters using classical equations (Bennett, M.V.L., Ann. N.Y. Acad. Sci., 137: 509-539, 1966). When the potential assymetry was attenuated and/or inverted by increasing the potential of the postsynaptic fiber, electrotonic transmission of positive currents from the postsynaptic cell was enhanced, but more surprinsingly, hyperpolarizing ones were also transmitted. Plots of junctional resistances R_{J+} and R_{J-} calculated from presynaptic injections of either sign, confirmed that the GMS became gradually bidirectional: both R_{J_s} were exponentially reduced to the same steady state value of 70 to 90 KΩ when the GMF was 40 mV more negative then the LGA. Because of LGA's delayed rectification, a similar range of ΔVs could not be fully obtained with current pulses through the postsynaptic electrode. Yet, addition of TTX (50 μM) and 4 AP (5 mM) to the perfusing solution allowed sufficient voltage displacements to demonstrate that, at least in a narrow range, junctional conductance was again enhanced, a small fraction of depolarizing currents now passing in the retrograde direction. Finally, modification of coupling following voltage changes was almost instantaneous, indicating that mechanisms underlying voltage-dependence of electrotonic coupling in this preparation are different from those involved at amphibian embryonic junctions (Spray, D.C., Harris A.L. and Bennett M.V.L., Science, 204: 432-434, 1979). In conjunction with other diverse behavior of bidirectional electrotonic synapses, this suggests that despite the apparent uniform structure of gap junctions, their permeabilities are not necessarily controlled in the same manner.

271.11 SINGLE NICOTINIC ACETYLCHOLINE INDUCED IONIC CHANNEL CURRENTS IN BULLFROG SYMPATHETIC GANGLIA. G. G. Schofield*, F. F. Weight, and M. Adler. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Single acetylcholine (ACh) current fluctuations were studied by the gigaohm patch-clamp technique in explant cultures obtained from the IX and X sympathetic ganglia of the bullfrog (Rana Catesbeiana). The cultures were maintained in Lebovitz L 15 medium supplemented with 7% fetal bovine serum at 22°C for 4-6 weeks prior to use. Patch pipettes contained 0.5-1.0 μM ACh in standard frog Ringer's solution. At these agonist concentrations, simultaneous multiple channel openings were rarely observed. ACh current fluctuations were detected in over 70% of the cell-attached patches examined, suggesting that the ACh channels are widely dispersed. The current amplitudes conformed to a Gaussian distribution for a single homogeneous population. The ACh current-voltage relationship was linear between the resting potential (assumed to be -50 mV) and -100 mV, yielding a value of 30 pS for the single channel conductance. Reversal potentials obtained by extrapolation were close to 0 mV. Channel open times were predominantly single exponential functions of time at all membrane potentials examined, with a time constant equal to the mean open time. Departures from exponential distributions were occasionally encountered. No systematic alterations in channel opening frequencies or open state dwell times were observed with changes in membrane potential between -50 and -100 mV. Similarly, the channel lifetimes exhibited little voltage dependence, as previously observed for the excitatory postsynaptic current (EPSC) from acutely isolated bullfrog ganglia (MacDermott et al., J. Gen. Physiol., 75: 39-60, 1980). However, the mean lifetime of these ionic channels ranged from 10-40 msec at a membrane potential of -70 mV, which is somewhat longer than has been reported by MacDermott et al., for the EPSC time constant of decay in bullfrog sympathetic ganglia. By comparison, the single nicotinic ACh channel of vertebrate skeletal muscle has been found to have a conductance of 30-50 pS, an open time voltage-dependence of e-fold for 80-100 mV and a reversal potential of 0 to -15 mV. Our results suggest that the ACh channels in this cultured vertebrate neuron resemble nicotinic channels of skeletal muscle in their conductance and reversal potential but differ principally in that the ganglionic ACh channel lifetime has little voltage-dependence.

271.12 SOMA AND DENDRITIC SPINE TRANSIENTS IN INTRACELLULARLY STAINED HIPPOCAMPAL NEURONS. D. A. Turner. Institute of Neurophysiology, University of Oslo, Oslo, Norway.

'In vitro' hippocampal neurons (CA1 and CA3 pyramidal cells, dentate granule cells; n=4 each) were characterized (input resistance, time constants: τ_0, τ_1) and injected with HRP. The stained neurons were measured and approximated by a series of cylindrical segments (300-600), then corrected for 4% measured shrinkage. Laplace transform cable equations (Barrett and Crill, 1974) were applied segment by segment. Dendritic spines were included (3-13x10^3/cell), using EM dimensions corrected for 40% linear shrinkage. Input impedances, Z(frequency), were calculated for both the soma and dendritic spines. These Z(f) were converted to transient impedances, z(time), using a reverse Fourier transform. For a particular site, z(t) was convolved with a transient input to calculate the voltage response.

Somatic step current pulses were simulated and analyzed for τ_0 and τ_1. The τ_0/τ_1 ratios were not significantly different (comparing the means with an F test) for the observed (Obs) and calculated (Cal) transients, either within or between the 3 classes; overall averages were 9.6±4.0 (Obs) and 5.9±1.0 (Cal) (mean±SD). Another index of comparison was equivalent-cylinder (E-C) electrotonic length (L), computed from τ_0 and τ_1. The mean E-C L values were also not significantly different, either within or between cell classes, averaging overall 0.91±0.23 (Obs) and 0.92±0.10 (Cal). Using these two indices, the segmental cable model appeared to adequately recreate the observed transients.

Excitatory dendritic spine synapses (dentate and CA1 cells) were simulated with a smooth conductance change (fast input transient (α=50), equilibrium potential of 55 mV). 'Quantal' synaptic events on mid-molecular layer dentate dendritic spines were simulated (72 sites, 4 cells). An input transient which produced a direct dendritic spine response of 19.9±13.3 mV (charge non-linearity, compared to soma, of 29%) resulted in an indirect soma response of 100.5±85.9 μV, close to the observed value (McNaughton, Barnes and Andersen, 1981). This indirect soma transient possessed a time to peak of 0.47±0.23 τ and a halfwidth of 1.03±0.26 τ. The same input directly applied to the soma produced a peak of 717 μV and injected 6.7x10^{-14} C of charge. Contrary to predictions, no modulation of transient inputs due to dendritic spines could be identified for these neurons.

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272.1 FIRING RATES OF MOTOR UNITS DURING MVC OF DIFFERENT HUMAN MUSCLES. F. Bellemare*, S. Smith*, J.J. Woods*, R. Johansson * and B. Bigland- Ritchie. J. B. Pierce Foundation. and Quinnipiac College, New Haven, CT. 06519.

Using conventional recording electrodes it is seldom possible to measure with certainty the firing rates of individual motor units during high force contractions owing to interference from other surrounding active units. We have used tungsten microelectrodes with an uninsulated tip of 10-15 µm (diameter 0.2µm), to record single unit activity from various human muscles during maximal voluntary contractions (MVC). After suitable manipulation, trains of regular spikes with a high signal to noise ratio may be obtained. Their amplitude, duration and shape are those of potentials from single muscle fibers(Fig. 1A & B). In 10s MVCs of the adductor pollicis muscle about 200 units from 4 subjects had a mean discharge rate of 28.2 ± 6.4 Hz. In similar experiments on biceps brachii the corresponding rates were 35.1 ± 8.3 Hz (N = 182). The higher mean rate and greater range of discharge rates for biceps brachii (Fig. 1C) may reflect its higher proportion of fast twitch fibers. (Add. Poll. ≅ 20% FT; Biceps ≅ 50% FT). Other muscles are being examined.

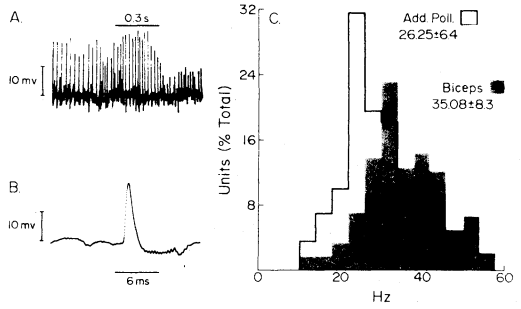

Fig. 1. A). Fast spike train from biceps (50 Hz). Rate measured during 0.3s bar; B). Single potential from train in A; C). Histograms in mean firing rates for adductor pollicis (N = 199) and biceps brachii (N = 182) units.
Supported by USPHS Grant NS-16338.

272.2 CHANGES OF SINGLE MOTOR UNIT FIRING RATES DURING FATIGUE AND AFTER POST FATIGUE ISCHEMIA. B. Bigland-Ritchie, J. J. Woods*, S. Smith*, F. Bellemare*, R. Johansson and O.C.J. Lippold*.(Spon: J. Stitt). John B. Pierce Foundation and Quinnipiac College, New Haven, CT. 06519.

Single motor unit potentials were recorded from the human adductor pollicis muscle using tungsten microelectrodes (see Bellemare et al, Neuro Sci, 1982. During brief (10s) maximal isometric voluntary contractions (MVC) executed once every 3 min. the average firing rates of about 200 units from 4 subjects ranged from 15-40 Hz, with a mean of 26.2 ± 6.4 Hz. When a maximum effort was sustained the mean value declined by about 25% after 30s, 40% after 60s, and 50% after 90s. Generally only short trains of potentials were recorded from each unit in order to sample a wide population. Occasionally single units were followed for up to 20s. For some the rates fell smoothly and rapidly (eg from 34 to 17 Hz in the first 15s). Others remained relatively constant. The reduction in mean motor unit spike frequency was accompanied by a similar, or greater, slowing of muscle speed (relaxation rate), suggesting that tetanic fusion is achieved in fatigued muscle at substantially lower excitation frequencies. Thus the reduction in discharge rates need not be responsible for loss of force.

When the muscle is kept ischemic following fatigue neither force nor relaxation rates recover. After 40s MVC the mean firing rate was 15.2 ± 2.2 Hz. This was followed by 20s anaerobic rest. In subsequent brief MVCs the mean motor units rate remained depressed, ie 14.8 ± 5.3 Hz. Periodic monitoring of the M wave evoked by single maximal shocks to the nerve throughout revealed no sign of neuromuscular conduction failure. Thus the motor unit potential frequencies correspond with motor neuron discharge rates, and suggest these may be regulated to match changes in the muscle contractile properties.

Supported by USPHS Grant NS-14756 and the Muscular Dystrophy Association.

272.3 EMG AND FREQUENCY ANALYSIS OF PHYSIOLOGIC ACTION TREMOR DURING ACUTE COLD STRESS. D. J. Howard* and R. S. Pozos, Dept. of Physiol., Sch. of Med., Univ. of Minn., Duluth, MN 55812.

An involuntary high frequency oscillation accompanies slow voluntary movements of the ankle (Pozos, R. S., Iaizzo, P. A., and Petry, R. W., J. Appl. Physiol. 52(1): 226-230, 1982) and has been called a physiologic action tremor (PAT). PAT may be a physiologic precursor to large amplitude oscillations such as physiologic clonus (Iaizzo, P. A. and Pozos, R. S., accepted in J. Appl. Physiol., 1982). The present study is a continuation of studies on PAT in which we investigated whether PAT also occurs during voluntary oscillation of the wrist and whether PAT is a precursor to shiver. The experiment was designed to test whether the amplitude of PAT would be altered previous to an overt oscillation (shiver) of the forearm. Surface EMGs were recorded from the extensors and flexors of the wrist while acceleration was measured using an accelerometer taped to the dorsum of the hand. The experimental design consisted of having measurements taken with the subject in a room with a temperature of 75°F (24°C) and then placed in a cold environmental chamber with a temperature of 35°F (2°C). In both situations, the subjects were asked to slowly (.5-1 Hz) extend and flex their wrist. EMG and acceleration data were recorded on a Hewlett Packard tape recorder and later analyzed on a MINC-11 computer. All measurements were made before there was any pronounced amplitude modulation of the EMG signal, which we considered to be a frank shiver state. While in the cold room, a rectal thermocouple monitored core temperature.

Six subjects (3 female, 3 male) demonstrated a PAT of the wrist with a frequency of 10 Hz ± 1 Hz and an acceleration range of 280-665 cm/sec^2. In the acute cold experiment there were two kinds of responses which occurred. In one group there was a 2-fold increase in the amplitude of PAT while in the other group there was a corresponding decrease of similar magnitude. Phase analysis between the major PAT frequency and corresponding frequency in the extensor EMG indicated a 20° phase increase in those in which the amplitude of PAT increased whereas there was a 30° phase decrease in those in which the amplitude of PAT decreased.

Collectively this data documents that there is a physiological action tremor of the hand whose amplitude is significantly altered by acute cold exposure previous to the presence of shiver. In none of the subjects was there a decrease in core temperature so that the changes in the amplitude of PAT seem to be dependent on sensory input.

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272.4 RESPIRATORY MOVEMENT CONTROL DURING SPEECH: EVIDENCE FOR MOTOR EQUIVALENCE. C.J. HUNKER* and J.H. ABBS*. (SPON: M. Gibson) Speech Motor Control Labs., Waisman Center, Univ. of Wisconsin, Madison, WI 53706

Because the speech motor mechanism consists of several systems (e.g., laryngeal, respiratory, orofacial), coordination across these semi-independent systems as well as among the components within a system is not trivial. Relevant to this problem is the neural control process superimposed over several potentially independent movements to ensure their complimentary contribution in achieving common goals. The operation of motor equivalence implies that there are several levels of motor programming; the more general "higher" levels are not involved with control of detailed subcomponents. Several studies support motor equivalence coordination in the limbs & in orofacial speech movements. The control of the respiratory system for speech may operate similarly, given it's multiple degrees of freedom in producing speech alveolar air pressures. That is, alveolar air pressure can be produced by many different combinations of recoil and active muscle forces. Similarly, a given lung volume can be produced by many different combinations of rib cage and abdominal movements.

To evaluate respiratory control, movements of the rib cage (RC) and abdomen (AB) were transduced along with alveolar pressure and lung volume. By recording these variables for repetitions of the same speech sequences, it was possible to observe subgesture variability where equivalent lung volumes and/or equivalent alveolar pressures were achieved. The data obtained suggested motor equivalence at several levels in respiratory motor control during speech: (1) when initial lung volumes were equivalent, RC and AB positions varied reciprocally in their common contributions to those lung volumes; (2) when initial lung volumes were unconstrained, they varied considerably, requiring reciprocal muscle adjustments to offset the varying recoil forces in achieving equivalent alveolar pressures; (3) when expiratory lung volume trajectories for a sentence were equivalent, relative contributions of the RC and AB varied considerably throughout these trajectories. These data demonstrate that speech respiration is not patterned stereotypically. Rather, motor equivalence coordination permits, with apparent ease, substantial flexibility in the detailed patterns producing these complex voluntary behaviors.
Research supported by NIH grant NS-13274-06.

272.5 A CLOSE RELATIONSHIP BETWEEN WHOLE MUSCLE EMG AND THE RECRUITMENT AND FIRING RATES OF SINGLE MOTONEURONS. N. Sugano*, W.B. Marks, J.A. Hoffer, J.W. Fleshman, and G.E. Loeb, Laboratory of Neural Control, NINCDS, Bethesda, MD 20205

The firing rates of single motoneurons recorded in cat L5 ventral root during normal locomotion tend to be highly modulated rather than fixed to some "preferred discharge rate" (Science 213:466, 1981). We have fit motoneuron activity to a simple function of the parent muscle EMG recorded by implanted bipolar electrodes: $R(t) = k(E(t) - T); \quad R > 0$

where R is the instantaneous firing rate, E is the rectified, filtered whole muscle EMG (38 msec time constant), T is the threshold EMG level at which the unit is recruited, and k scales EMG voltage to firing rate. Twenty-five units from the quadriceps and sartorius muscle groups have been so fit. The general finding is that this function accounts for 90-98% of the variance of unit activity and that k and T vary little over the entire range of walking speeds. In particular, even brief fluctuations in EMG envelope from step to step are reflected in single motoneuron activity, suggesting that these fluctuations result from changes in a central driving function which is common to the entire motoneuron pool rather than from random changes in the electrical coupling or waveform occlusions picked up by the EMG electrodes.

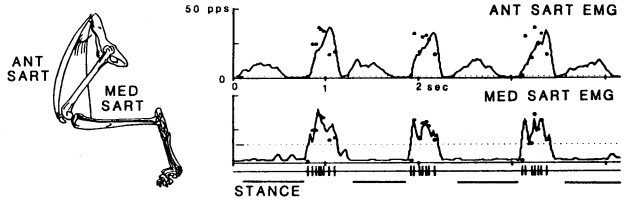
To further demonstrate this relationship, we used EMG envelopes as templates for depolarizing currents which were applied intracellularly to lumbar motoneurons in an acute spinal preparation. By adjusting the resting membrane potential (corresponding to T) and the scale factor for voltage to current (corresponding to k), we caused single motoneurons to generate spiking activity which corresponded almost exactly with the patterns originally recorded from motoneurons when the EMG record was obtained. Furthermore, a motoneuron could be made to generate activity patterns resembling either early recruited (presumably slow) or late recruited (presumably fast twitch) units by adjusting the two parameters.

In multifunctional muscles such as sartorius, individual motor units participate in only one of the different periods of EMG activity in each step cycle, suggesting that there are different motor pools for each function (see adjacent poster by Hoffer et al.). Within each period of unit activity, the fitting function works as well as for unifunctional muscles.

These results suggest that whole muscle EMG can provide accurate and quite detailed information about the net input to a motoneuron pool. In this situation, we found no need to postulate any special pathways for recruitment of the different motoneurons activated during walking, which appear to differ only by their intrinsic threshold and synaptic strength from common sources controlling the entire pool during this task.

272.6 CAT SARTORIUS: THREE FUNCTIONALLY DISTINCT MOTONEURON POOLS SUPPLY A SINGLE MUSCLE. J.A. Hoffer, N. Sugano*, W.B. Marks & G.E. Loeb. Laboratory of Neural Control, NINCDS, Bethesda MD 20205.

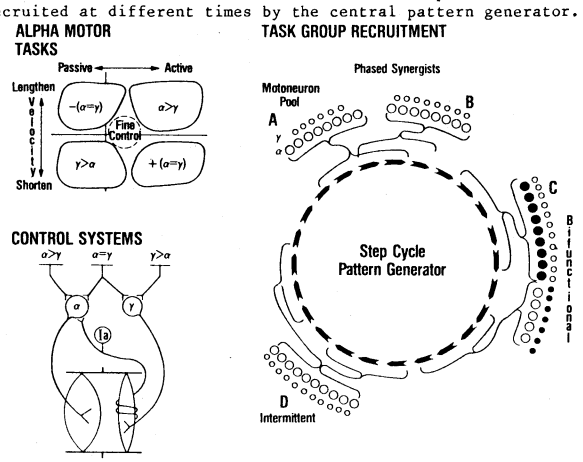
Recordings from motoneurons in walking cats have shown that firing frequencies are highly modulated. In general, a unit's frequencygram can be closely fit by a simple function of its parent muscle EMG (see adjacent poster by Sugano et al.). This approach, which renders a measure of the correlation between the instantaneous level of activation of a single unit and that of the rest of the pool, has provided suggestive evidence that the sartorius muscle is supplied by three separate motoneuron pools.



From anatomical considerations (see diagram) sartorius in cat is mainly a hip flexor, with additional mechanical action on the knee which is complicated by a distributed insertion. The anterior portion (ANT SART) pulls on the patella and thus extends the knee, whereas the medial portion (MED SART) inserts along the tibia and thus flexes the knee and rotates the leg. EMG patterns recorded from ANT SART and MED SART during locomotion differ. ANT SART shows two bursts of activity per step cycle which are generated by two subpopulations of motor units, one being active only during flexion, the other only during extension (Hoffer et al., J. Physiol. 308:20P, 1980). MED SART shows only one period of EMG activity, during flexion. The EMG bursts in MED SART begin sooner, peak earlier, and differ in shape from the flexor bursts of ANT SART. For six recorded sartorius flexor units the frequencygrams have been fitted by the EMG profile of one region of the muscle more precisely than by the other (one unit's frequencygram is shown along with the best fit provided by each EMG). This suggests that the "flexor" motoneurons that supply MED SART belong to a separate pool from the motoneurons that generate the "flexion" burst in ANT SART. We submit that sartorius motoneurons are divided functionally into three pools that act separately during locomotion to perform three tasks: knee extension (E_{2-3} phase, stance); knee and hip flexion (F phase, early swing); hip flexion and knee extension (E_1 phase, late swing).

272.7 TASK GROUPS - A PROPOSED FUNCTIONAL UNIT FOR MOTOR CONTROL G.E. Loeb, Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205

There is growing evidence that individual muscles are each heterogeneous collections of motor units and proprioceptors with different physiology and functions. There is no a priori reason why the gross anatomical decision to give a piece of mesenchymal tissue a distinctive name should reflect its neural control; therefore, we propose a new functional unit which better reflects the various tasks which the muscles perform. We have elsewhere reported data indicating particular uses of parts of the afferent and efferent apparatus to perform different tasks, where the distinction may relate to whether the tasks are performed with the active muscles shortening or lengthening. We here generalize that notion by defining a Task Group's contribution as an impulse to maintain (against gravity) or change momentum of the limb, i.e. the product of force and time, independent of length. A given muscle's velocity is better seen as the result rather than the cause of the necessary skeletal movement of locomotion. This velocity determines the appropriate control system, giving rise to the need for multiple control systems within some multifunctional muscles. For example, bifunctional muscle C below is used during an unloaded contraction ($\gamma > \alpha$ task domain) and an active "lengthening contraction" ($\alpha > \gamma$ task domain). Maintenance of useful Ia feedback signals requires the two different control systems shown below, which appear to be embodied in two subdivisions of the motoneuron pool which are recruited at different times by the central pattern generator.



272.8 HUMAN EMG OPERANTLY CONDITIONED AT DIFFERENT POINTS IN THE WALKING CYCLE. M. C. Wetzel, L. K. Gorman* and W. G. Himadi*. Psychol. Dept., Univ. of Ariz., Tucson, AZ 85721.

A major but incompletely studied cause of human and animal locomotion is provided by the discriminative stimuli of operant conditioning (Wetzel, M.C., J. Human Movement Studies, in press, 1982). Work reported here compared, across five temporal positions of the step cycle, strength of discriminative stimulus, Sd, control exerted by a light flash over integrated surface EMGs of the rectus femoris, RF, muscle. Persons walked on a motor-driven treadmill. Under computer programming initiated by a heel switch, once in each cycle either a green light required a 100-400 msec burst of preset threshold amplitude within 700 msec, or else a red light required muscle silence. A high or low tone indicated success or failure, respectively. In early stages of training, trials (10 consecutive cycles) consisted of systematic sequences of green and red lights, for a given step cycle position, while in later stages random sequences were generated.

At every point in the step cycle a large burst of activity in RF, a knee extensor and hip flexor, was conditioned. Step cycle position per se did not influence, to an appreciable extent, total number of trials to criterion, defined as two consecutive successful trials of 10 steps each. Effective balance was maintained throughout. For a given cycle position, errors did not decrease gradually as a function of experience, nor was there a steady decrease across positions. Instead, for each position the criterion was met abruptly, sometimes after several tens of trials but often after only a few trials.

Since the green light altered greatly the usual slight activity of the RF muscle that is seen when subjects are just asked to walk comfortably on a treadmill, the existence was ruled out of an obligatory pretouchdown extensor burst or any other strongly competing reflexive or acquired behavior. It was suggested that the powerful, successful control had been achieved by an Sd complex composed of both the light flash and associated movement-produced stimulation. Proprioceptive and cutaneous afferentation was most plausibly engaged in discriminative control when a burst slightly preceded the light, as was seen occasionally, but local afferentation also was assumed to add to the light's effectiveness at all temporal positions of the step cycle.

272.9 DIFFERENTIAL ACTIVITY OF INNERVATION SUBCOMPARTMENTS OF CAT LATERAL GASTROCNEMIUS DURING NATURAL MOVEMENTS. C.J. Russell, D.C. Dunbar*, D.S. Rushmer, J.M. Macpherson, J.O. Phillips*, Good Samaritan Hospital, Portland, OR 97210.

The reported compartmentalization of the innervation of hindlimb musculature (English, A.W. and W.D. Ledbetter, Am. J. Anat., in press) raises the question of whether this compartmentalization has functional correlates.

In an attempt to answer this question for one specific muscle, we implanted separate pairs of EMG electrodes in three of the four innervation subcompartments of lateral gastrocnemius muscle in cat. Proximally, three of the compartments correspond roughly to the three grossly distinguishable proximal heads of the muscle, the lateral (LGSL), medial (LGSM), and intermediate (LGSI) heads. To take advantage of this arrangement, the electrodes were implanted one-third of the distance distal from origin to insertion. EMG responses were recorded simultaneously from these three electrodes and from electrodes implanted in other hindlimb muscles during controlled postural perturbations. When the raw EMG responses were viewed during perturbations and during quiet standing, all three electrodes showed multiple-unit activity, indicating that the integrated EMG response is likely to be representative of the activity of that head rather than of the activity of aberrant single units. Myotatic responses evoked by a low-amplitude step rise to the implanted hindlimb support were of equal latency (10 msec), with thresholds of approximately one mm displacement at 20 mm/sec.

During forward translation of the entire body, which loads the hindlimbs, the ankle joint is stabilized by two separate groups of muscles which are active at different times. LGSM functions with the first group while LGSI and LGSL function with the second group of muscles. During contralateral anterior limb drop, the forelimb is suddenly unloaded, causing unloading of the implanted hindlimb. In contrast to the previous perturbation, LGSI acts with one group of muscles while LGSL and LGSM act with a second group.

These data indicate that the subcompartments of cat LG can be activated independently by the CNS during natural movements. The movements in question are automatic and highly stereotyped, and it remains to be determined whether similar patterns of independent activation are seen during "voluntary" as opposed to "postural" movements.

Supported by MRC of Canada and Oregon MRF.

272.10 INTRACONTRACTILE LENGTH CHANGES IN THE PROXIMAL AND DISTAL COMPARTMENTS OF THE SEMITENDINOSUS. S.C. Bodine*, R.R. Roy, R.F. Zernicke, and V.R. Edgerton. Dept. of Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

The cat semitendinosus (ST) has an unusual architecture for hindlimb muscles in that it is divided into two distinct " in-series" compartments by a connective tissue band with each having a separate innervation (Bodine et al., J. Neurophysiol., 1982). The fibers in the distal compartment (STd) are twice as long as those in the proximal compartment (STp), and thus the STd has a greater compliance than the STp. During locomotion, the ST is active during hip extension and knee flexion and exhibits two EMG bursts during each step cycle. Although independent activation of each compartment is possible, it appears that both compartments are activated simultaneously during locomotion (Murphy et al., Med. Sci. Sports Exercise, 1981).

Contractile properties of each compartment were studied in an in situ preparation under three stimulation conditions: (1) STp alone, (2) STd alone, and (3) STp and STd simultaneously. To determine length changes within each compartment, length excursions were filmed at 200 fr/s during isotonic contractions at various loads.

When both STp and STd were stimulated simultaneously, the two compartments responded differently depending on the loading condition. When the ST contracted against low loads (high velocity) both STp and STd shortened. However, relative to their original length, the STp shortened twice as much as STd. Under high loads (low velocity), STp shortened whereas the STd lengthened. When only the STp was stimulated, STp shortened approximately the same amount as when both STp and STd were stimulated simultaneously. In comparison, when STd was stimulated alone, STd shortened nearly twice as much as when both were stimulated. When each compartment was stimulated separately under similar loading conditions, STd shortened twice as much as STp. However, when both STd and STp were stimulated simultaneously the STd predominated; the STd shortened half as much as when it was stimulated alone, whereas STp shortened the same amount when independently or simultaneously stimulated.

These data demonstrate how a marked difference in the nature of the afferent input from each compartment could occur under different loading conditions. But in spite of that, the Ia input into the ST motoneuron pool is similar to both the proximal and distal motoneurons (Nelson and Mendell, J. Neurophysiol., 1978). The difference in the afferent input from each compartment should be very similar. These differential dynamic effects demonstrate the need for a better understanding of the level of synchronization of the multiple EMG bursts found in the ST during normal locomotion. (Supported by NIH Grant NS16333)

272.11 STANCE POSTURE CONTROL IN SELECT GROUPS OF CHILDREN WITH CEREBRAL PALSY: DEFICITS IN SENSORY INTEGRATION AND MUSCULAR COORDINATION. L.M. Nashner and A. Shumway-Cooke. Neurological Sciences Institute of Good Samaritan Hospital and Medical Center, 1120 N.W. 20th Avenue, Portland, OR 97209. This study has focused upon the automatic components of posture and movement in a group of ten cerebral palsy children carefully selected to represent a spectrum of abnormalities relatively pure by clinical standards (three spastic hemiplegics, three ataxics, three spastic diplegics and one athetoid) and ten age-matched normals. Each subject stood unsupported upon a movable platform and within a movable visual surround and was then exposed to external perturbations or was asked to pull with one arm upon a movable handle. In comparing the performance of cerebral palsy children in each clinical category with the age-matched normals and with normal adults assessed in previous studies, the process of maintaining stance was subdivided into two component functions: substrates which determined the onset timing, direction, and amplitude of postural actions termed "sensory integration", and those establishing temporal and spatial patterns of muscular contractions appropriate to produce effective movements termed "muscle coordination". Among seven of the ten cerebral palsy children we found a clear localization of dysfunction within either sensory integrating or muscle coordinating mechanisms. Specifically, muscular coordination abnormalities were characterized by disruption of the temporal sequence of activation, which in normals began at base of support, and by breakdowns in the spatial structure of synergist contractions, which in normals were characterized by fixed ratios of contractile strength. In contrast, children with abnormalities limited to sensory integration responded to perturbations much more slowly and under conflict conditions inappropriately, and yet the temporal-spatial structure of their contractions was always within normal limits. These results are providing some new insights into the organizational principles subserving muscular coordination and sensory integration as well as suggesting methods for developing a more systematic understanding of the abnormalities of movement control.

272.12 VISUAL AND VESTIBULAR CONTRIBUTIONS TO LANDING FROM JUMPS IN CAT. P.A. McKinley [1], & J.L. Smith. Neuromotor Control Lab & Brain Research Institute, UCLA, L.A. CA 90024

In a previous report (Neurosci Abst., 1980), data were presented suggesting that forelimb EMG is predetermined centrally for voluntary landing. The onset of prelanding elbow extensor EMG was shown to be constant across all jump heights with reference to landing and not to take-off.

The following study assessed prelanding EMG responses in elbow extensors during landing from jumps in normal blindfolded (BF) cats and labyrinthectomized (L) cats with and without vision occluded. Five trained normal adult cats were allowed to jump several times at 0.6m before BF. Subsequently the animals jumped 3 more times at 0.6m,0.8m, 1m & again at 0.6m. 2 L cats jumped only at 0.6m. Extensor EMG was coordinated with high speed film.

In the BF cat, 4 distinct prelanding patterns could be distinguished: Normal (N), a typical 2 burst response characteristic of a jump with vision, as extensor onset began 70 ± 8ms before landing; Early (E), a normal 2 burst pattern that occurred too soon with reference to landing; Continuous (C), a constant EMG throughout the entire prelanding period; Delayed (D), an absence of EMG prior to landing. Jump down conditions determined which type of response was elicited. When the height could be anticipated, an N response occurred. When the animal was 'tricked' by an unexpected change in jump height, the cat displayed a pattern of EMG activity appropriate for the jump height just previously experienced, not for the actual height; an increase in jump height elicited an E response, while a decrease elicited a D response. If jump height was uncertain, the cat commonly exhibited a C type.

In the presence of visual cues, L cats executed jump downs, demonstrating an N response although prelanding IEMG was less than that of control jumps. In addition, landing was awkward as the forelimbs collapsed and the ventral surface of the trunk contacted the landing pad. Over time, prelanding IEMG increased and landing occurred without collapse of the forelimbs. Without visual cues, these cats were unable to execute a jump.

These results suggest that visual input may normally regulate timing of the motor program and dominate vestibular reflexes. Conversely, vestibular input may be primarily manifested as gain in extensor activity rather than in temporal sequencing. When *visual input is absent and jump height uncertain, vestibular input* becomes more influential in determining the pattern of prelanding activity.

Supported by NIH grant NS 16333

1 The author was previously published as P.A. Reback

272.13 DISSOCIATION OF PITCH AND YAW COMPONENTS OF A SOMESTHETIC ORIENTATION-LOCALIZATION MOVEMENT AFTER CORTICAL DAMAGE.
R. B. Glassman. Dept. Psychol., Lake Forest College, Lake Forest, IL 60045.

This abstract relates after-the-fact theoretical considerations to empirical observations, surprising at the time, that occurred while studying cutaneous behavior in cats.

Theory: To build the simplest conceivable neural system to operate an organism having y muscle groups, each of which participates in moving the mouth to any of x discriminable areas on the body surface, there must be at least xy neural connections. A somewhat more complex structure, with a small number (n) of variably excitable central associative functions would require only nx + ny neural connections (and, moreover, might be an evolutionary step towards a system with capabilities for learning and modulation by motivational states). The associative functions might be analogous to those that control industrial "robot" arms, which can reach any point in a volume if they have one joint with two articulations and an additional joint with one articulation (Engelberger; Robotics in Practice; London: Kogan Page, 1980). It seems possible that a cat may be able to "rough out" an orientation towards any point on its own body surface primarily by setting movement parameters of pitch, yaw, and the anterior-posterior location of spinal bending; thus n=3. (This analysis ignores the sequential patterning of a movement and feedback corrections.)

Data: Blindfolded cats with unilateral cortical damage were observed for speed and accuracy of localization of points on the body that were touched with a piece of meat. Five animals were sometimes clearly observed to complete a downward movement (pitch) to stimulation of the paw contralateral to damage, before initiating any lateral component (yaw). Three cats, on the other hand, turned laterally on some occasions well before achieving accurate pitch. These latter animals had less severe damage to SII and insular cortex than did the former and they had a lesser overall tactile deficiency. (See Glassman & Glassman, Physiol. Behav., 1977, for earlier findings about cortical localization of somatosensory behavioral functions in cats.)

It will be argued that yaw is controlled by the balance of inputs to the left and right hemispheres, while pitch depends on topographic details of central somatosensory maps. Location of spinal bend must also depend on topographic details but empirically anterior-posterior localization appears less refined in cats than is proximal-distal localization (pitch). One implication is that receptive fields in the orientation-localization system might have smaller vertical than horizontal dimensions.

272.14 SOME VISUAL, EFFERENT, AND SUBSTRATE OF SUPPORT INFLUENCES IN THE CONTROL AND APPRECIATION OF MOVEMENT AND SPATIAL STABILITY.
P. A. DiZio* and J. R. Lackner. Psychology Department, Brandeis University, Waltham, MA 02254.

We varied the relationship among visual motion, substrate motion, voluntary stepping direction, and body displacement using an optokinetic drum, platform, and handle bar coaxial and separately rotatable. 12 subjects (Ss) participated. After 3-37 sec, when

1) Stepping in place (SIP), platform-moving, drum-stationary: some Ss (50%) experience self motion in the direction of stepping with platform stationary and drum moving with them.

2 & 3) Walking forward (WF) platform-stationary, drum-stationary or SIP, platform and drum moving together in the direction opposite stepping but at the same speed: Ss experience WF, platform stationary, drum-stationary.

4) SIP, platform-moving, drum-moving in the same direction and at twice platform velocity: all Ss experience drum stationary and their bodies being displaced at a velocity equal to but opposite in direction to actual drum velocity; some Ss (50%) experience platform stationary and either their stepping rate to have doubled or the apparent length of their legs to have increased; remaining Ss experience platform moving in the direction of their apparent walking.

5) SIP, platform and drum rotating in opposite directions: Ss experience self-displacement in direction opposite drum motion, perceive drum stationary; some Ss(37%) experience a reversal in the direction of their actual stepping movements, others (42%) experience their steps as propelling them in the wrong direction.

Electroculograms showed that eye movements were primarily determined by optical flow patterns although occasionally nystagmus was elicited during apparent self motion when the optokinetic drum was stationary (condition 1 above) or was determined by the direction of stepping rather than optical flow (condition 5).

These results complement earlier observations (Lishman, J.R. and Lee, D.N. Perception, 2, 1973; Bles, W. and Kapteyn, T.S. Agressologie, 18:6, 1977; Lackner, J.R. and DiZio, P.A. In Sensory Experience, Adaptation, and Perception, Spillmann, L. and Wooten, R.A., Eds., Erlbaum Assoc., In Press.) The present findings are inconsistent with linear sensory-convergence models of orientation. Instead, they emphasize that the control and appreciation of movement and orientation are under multimodal sensory and efferent influence. They indicate that the representations of our body motion, our body dimensions, our substrate of support, our visual world, our willed actions, and the apparent consequences of our willed activity are perceptually labile and subject to remappings during exposure to sensory motor discordances.

Supported by NASA contract NAS9-15147.

272.15 POSTURAL AND EMG REACTIONS OF STANDING HUMAN SUBJECTS TO PERTURBATIONS OR VOLUNTARY MOVEMENTS OF THE ARM IN THE FRONTAL PLANE.
M.R. Zomlefer, C.C. Boylls, and R.W. Angel. RERanD Center, VA Medical Center, and Dept. Neurology, Stanford University School of Med., Palo Alto, CA 94305.

Studies of postural stabilization associated with upper-limb movement in standing humans have tended to stress performance in the antero-posterior plane. We recently have begun to examine whether conclusions drawn under these conditions generalize to frontal-plane arm motion requiring stabilization of lateral sway. To simplify the mechanical analysis, subjects are fitted with casts restricting motion of the right arm primarily to the shoulder and fingers. They then are asked to stand quietly, barefooted and with eyes closed, upon an instrumented force plate where two experimental maneuvers are performed. The first requires rapid voluntary raising of the arm to shoulder height in the frontal plane following an auditory cue. The second employs counterweights which elevate the arm to the shoulder-height position and allow either transient or sustained torques to be applied at the shoulder. EMG activity accompanying the lateral sway reactions is simultaneously recorded from the medial deltoid of the casted arm, from the ipsi- and contralateral lumbar back musculature, and from the femoral abductors and adductors of both legs.

In general support of the idea that "postural pretuning" precedes voluntary movement, we have seen that EMG activity in the contralateral lumbar back muscles appears 30-60 msec in advance of the earliest medial deltoid bursts during rapid lateral arm-raising. Activity alterations in the ipsilateral leg (both adductors and abductors) can also occur prior to the deltoid burst, while changes in the contralateral leg appear 50 msec or more thereafter-- perhaps producing "compensatory" postural adjustments. The adductor/abductor interplay in each leg has a detailed structuring presently under investigation. EMG behavior during sudden loading of the deltoid with our counterweighting system produces prompt (20-30 msec latency) responses of the muscle, followed almost immediately by contraction of the contralateral lumbar back musculature not unlike what is seen during voluntary deltoid usage. However, back activity never appears to precede deltoid; no "preparation" for deltoid's response is evident. Very late (>150 msec post-perturbation) reactions sometimes appear in the contralateral femoral adductors. We have also looked cursorily at the primary ankle muscles (triceps surae and pretibial groups). However, these appear to have no participation in the preparation for frontal-plane arm movement. Results of the ground reaction-force analysis and of other frontal-plane movement situations are presently being studied.

272.16

WITHDRAWN

272.17 MUSCULAR CONTROL OF A LEARNED MOVEMENT. R.M. Enoka. Department of Physical Education, Univ. of Arizona, Tucson, AZ 85721.

An attempt was made to identify the invariant characteristics of a learned movement that comprised sequences of shortening-lengthening muscle contractions. Six competitive weightlifters, 3 of whom were more skilled than their counterparts, were each tested on 2 occasions. A testing session included 8 lifts, 5 with expected loads and 3 with unexpected loads. Relative to a coach-specified "maximum" effort (100%), each session comprised 1 lift at 80%, 1 at 90%, 3 at 100% and 3 at either 95 or 105%, the latter 3 alternating between sessions. At the time of testing, the 100% loads represented 86±3 and 86±4% ($p<.10$) of the estimated maximum capabilities for the skilled and less skilled groups, respectively. Each lift was filmed (~100 Hz), EMG recorded for vastus lateralis (VL) and biceps femoris (BF) and the ground reaction force measured (sampled at 500 Hz). The cinematographically derived position-time records were filtered, segmental acceleration-time histories obtained by double differentiation and Newtonian equations of motion used to calculate the resultant muscle torques (RMT) between body segments.

The analysis focused on the portion of the lift in which the barbell was displaced from the floor to approximately waist height. Over this range of motion the "dominant" musculature has been demonstrated to be that about the knee joint, the angular displacement about which comprised two periods of extension, separated by an interval of flexion. The EMG profiles for VL and BF indicated that both muscles were active throughout the movement. The RMT-time histories were characterized by alternating periods of net extensor and flexor torques that were accomplished by epochs of shortening and lengthening muscle contractions.

As a test of the "speed control system" hypothesis (Freund & Büdingen, Exp. Brain Res. 31:1, 1978), the movement was examined in terms of the duration and intensity of net muscle activity. For the initial extensor torque epoch the analysis revealed no significant difference ($p=.78$) among the mean durations (.308, .322, .328 s) for the expected loads (80, 90, 100%) of both groups of subjects. For the subsequent flexor epoch, however, the mean durations (.160, .241, .270 s) were different, with the direction of the change depending upon the level of expertise. The mean torques for both the extensor (49.8, 51.3, 58.0 N.m) and flexor (15.7, 25.7. 30.5 N.m) epochs differed significantly across loads, i.e., average RMT covaried with load. With respect to the 100% load, the 5% perturbation did not significantly influence either the duration or intensity of the net extensor and flexor epochs. The control of multi-directional learned movements, therefore, appears more involved than the mere specification of average movement speed. [Supported in part by BSSG 61-2300 and RR-07096 NIH awarded to the Dept. of Kinesiology at the Univ. of Washington.]

272.18 CHEST SURFACE RECORDING OF DIAPHRAGM ACTIVITY IN MAN. R. Lansing and J. Savelle.* Dept. of Psychol. Univ. of Arizona, Tucson, AZ 85721.

Diaphragm EMG can be recorded non-intrusively from skin electrodes applied to the anterolateral chest surface where the muscle is closely apposed to the chest wall (See Derenne, Macklem, and Roussos, Amer. Rev. Resp. Dis., 1978). This would be the method of choice when oesophageal electrodes were impractical or when the costal diaphragm must be studied, but there is uncertainty about optimal electrode positions, the degree of intrusion of other respiratory muscles, and the effects of change in diaphragm position.

We studied EMG potential gradients with bipolar recordings from a chain of electrodes along the midaxillary line: 5th interspace to the pelvic crest. Control records were obtained from parasternal, pectoral, and abdominal sites. We measured airflow, volume, and pressure at the mouth, and abdominal circumference with a pneumograph. Eight healthy male subjects were studied in a relaxed, supine position. They ranged from slim to medium body build (weights: 133 to 207, skin-fold thickness over the lateral chest: 8 to 18 mm). Band electrodes (2 by 4 cm strips of silver coated fabric) reduced the intercostal activity (by cancellation) while still recording from the deeper lying diaphragm. The voltage patterns could be observed in the raw inkwriter records and were measured by ruler and planimeter.

EMG envelopes, synchronized with inspirations, were found at each midaxillary site: peak voltage was located at the 7th and 8th interspace bipolar pair, with progressively diminishing voltages above and below. During quiet breathing the point of maximum voltage averaged 20 uV and varied from breath to breath, paralleling volume changes. Increased inspiratory efforts, deep breaths up to 70% vital capacity and static efforts up to -40 cm H20, increased the voltage peak to 55 and 60 uV, respectively, with relatively small increases in voltage in the upper and lower interspaces and little at the pectoral and abdominal sites. The same gradients were found for subjects trained to inspire with abdomen moving out and little chest movement. For some subjects the voltage peak shifted down one interspace near the end of a deep breath reflecting diaphragm descent. More extreme inspiratory efforts, postural change, yawning, coughing, and expiratory efforts produce distinctive, easily recognized distortions of these voltage gradients.

We conclude that chest surface recording with an appropriate electrode array permits the identification of diaphragm activity by its unique surface patterns, and provides an estimate of the source and degree of contamination from other muscles. The inspiratory voltage profiles above are compatible only with a diaphragm source.

272.19 TRANS-SEGMENTAL RESPONSES OF MUSCLE NERVES SUPPLYING LATERAL LONGISSIMUS. S. Schwartz-Giblin, M. Halpern[1] and D.W. Pfaff. Rockefeller Univ., New York, NY 10021. [1]SUNY Downstate Medical Center, Brooklyn, NY 11203.

Lateral longissimus muscle in the rat is a prominent dorsiflexor of the lumbar spine which is active during walking, hindlimb standing and during cutaneously-evoked rump elevation associated with the lordosis reflex in reproduction. The muscle is innervated by the lateral branches of the dorsal rami of lumbar spinal nerves (Brink and Pfaff, Brain Behav. Evol. 17:1-47, 1980).

We have begun to study the segmental organization of the motor outflow to lateral longissimus with respect to muscle and cutaneous inputs in urethane anesthetized rats (1.4 gm/kg). Individual lateral nerve branches of the L3, L4 and L5 dorsal rami to lateral longissimus were dissected, ligated and cut peripherally. Bipolar teflon-insulated platinum hooks were used as stimulating or recording electrodes and were placed on each of the segmental nerves: either on the trunk proximal to branching, on a pure muscle branch, or on a musculocutaneous branch, i.e. one whose fibers exit through the aponeurosis to supply overlying skin. In some experiments, afferent conduction time to the cord was monitored with a silver ball electrode on intact dorsal roots. All exposures were covered with a Vaseline-mineral oil mixture.

Stimulation of a proximal trunk with single 0.1-0.2 msec cathode pulses averaging 50 μA (range: 12-275 μA) evoked multiple negative dorsal root potentials, the earliest of which had a mean onset latency of 0.8 msec (range: 0.5-1 msec, N=13) corresponding to an approximate conduction velocity of 43-50 M/sec. Infrequently, single current pulses within this range or greater evoked an early response in another segmental muscle branch at 1.5-2 msec latency which we presume to be monosynaptically relayed.

The most common response we recorded across segments in these experiments was a multi-unit response having a mean onset latency of 9 msec (range: 6-13 msec, N=21) which was followed occasionally by a second discharge after a delay of 40-60 msec. The response was predominantly evoked by stimulating a musculocutaneous branch with 2-3 pulses (1.3-4 msec pulse period) at currents averaging 7 x that required to excite the largest muscle afferents recorded in these experiments. At repetition rates of 1-2/sec the probability of response decreased after 30-60 sec and was often lost after 60-90 sec; it could be re-elicited at the same current strength after several minutes of non-stimulation.

The latency and lability of the response imply a multisynaptic central mechanism and suggest possible influences from suprasegmental pathways.

(Supported by NIH grants HD 13795 and HD 05751.)

272.20 IMPAIRMENT OR ABOLITION OF LORDOSIS RESPONSES TO SOMATIC STIMULI FOLLOWING TRANSECTIONS OF DORSAL OR LATERAL SPINAL COLUMNS IN THE SYRIAN HAMSTER. J. D. Rose and L. K. Smucker* Dept. of Psychol. Univ. of Wyoming, Laramie, WY 82071.

In the sexually-receptive female hamster, lordosis responses are elicited by light rapid brushing of hair within the lumbosacral region of the dorsal and lateral body surface. Since hair movement stimuli are known to be highly effective for activation of responses in dorsal column axons, effects of transecting the dorsal columns on elicitation of lordosis was examined. Transection of lateral spinal columns was employed in some animals as a comparison for effects of dorsal column cuts. Behavioral evaluation of lordosis responding before and after surgery was conducted in hormonally-primed ovariectomized hamsters by testing responses to males and to manual stimuli. Since the skin fields from which lordosis can be triggered extend very rostrally on the hamster's body, the spinal pathways were usually transected between the first and second cervical segments. Dorsal column cuts were bilateral but lateral column transections were usually unilateral due to the debilitating effects of such injury at the cervical level. The dorsal column transections produced either abolition of lordosis or a unique pattern of impairment in which the hamsters displayed hindlimb, rump and tail components of the lordosis response while failing to show immobility of the head, vibrissae and forelimbs. Lateral column damage sometimes abolished lordosis responses also, but in other instances was associated with a completely immobile lordosis posture which was asymmetric due to unequal muscle tone on the two sides of the body. Low dorsal column cuts at the thoracic-lumbar junction produced a different pattern of effects resulting in a spatial dissociation of the effective stimuli and motor responses. Stimulation of the upper lumbar and thoracic regions elicited head, vibrissae and forelimb immobility without rump and tail elevatior while perigenital stimulation evoked a response from the rump, tail and hindlimbs without engaging the head or forelimbs in the lordosis stance. The results clearly implicate the dorsal columr pathway in the somatosensory control of hamster lordosis and contrast with previous findings for the rat in which dorsal column cuts did not impair lordosis (Kow, L.-M. and Pfaff, D. W., Ann. N. Y. Acad. Sci., 1977, 290, 72-97). The results also suggest the presence of a dual control of lordosis in the hamster in which stimulation of skin trigger zones activates the response of the rump, tail and hindlimb components of lordosis through a lumbosacral spinal mechanism and a supraspinal mechanism is required for elicitation of immobility of the head, vibrissae and forelimbs.

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273.1 AXONAL AND DENDRITIC MORPHOLOGY OF TECTORETICULAR CELLS IN A TURTLE, PSEUDEMYS SCRIPTA. M. Sereno. Committee on Neurobiology, Univ. of Chicago, Chicago, IL 60637

The optic tectum projects to the reticular formation but little is known about the axonal and dendritic morphology of its output cells. Thus, solid-filled axons and cells were reconstructed from serial sections following tectal and tegmental HRP injections.

There are three tectobulbar pathways: a dorsal pathway (TBd) forms the contralateral predorsal bundle, an intermediate pathway (TBi) courses through the ipsilateral lateral reticular formation, a ventral pathway (TBv) courses along the ventral surface of the ipsilateral brainstem. TBd contains coarse and fine fiber components. Both have axons with rostrally directed ipsilateral collaterals arborizing extensively in profundus mesencephali rostralis (PMr), long rostral branches arising near the oculomotor complex and turning dorsolaterally into the ipsilateral entopeduncular nucleus, and laterally directed collaterals arising at regular intervals from main truncks in the predorsal bundle. TBi axons each emit a thick, rostrally directed branch that courses ventral to PMr. The main trunk then turns caudally to give off finer collaterals in profundus mesencephali caudalis (PMc) and at regular intervals in the lateral reticular formation. TBv has a fine ventrolateral component whose axons emit collaterals in the magnocellular nuclei isthmi, PMc, and locus coeruleus and then caudally in the neuropile at the ventral surface of the brainstem, and a medium caliber ventromedial component whose axons give off medially directed collaterals in the ventrolateral pontine tegmentum.

TBd injections label a uniform population of medium to large multipolar cells in the contralateral central gray. Their sparsely branched dendrites span up to 1 mm, often extending into the superficial gray, and bear fine spicules. Lateral tegmental injections label a heterogeneous population of ipsilateral cells. Large cells with several dendrites extending into the stratum opticum lie in and just above the periventricular cell layers. Large cells in the upper central gray have horizontally directed dendrites that turn vertically to reach the tectal surface with dendritic appendages in the central gray. Other central gray cells have vertically oriented dendrites bearing central gray dendritic appendages and an elaborate arborization just below the stratum opticum. These three cell types probably send axons into the TBi. Small cells in the superficial gray have dendrites with appendages in the central gray and a small dendritic arbor just beneath the stratum opticum. Small radial cells are labeled in the periventricular layers. These two cell types contribute to the TBv.

Thus, the topographic retinal input to the superficial gray layers of the tectum undergoes a complex, non-topographic transformation via the reticular formation output. (Supported by PHS Grant NS 12518).

273.2 TACTILE EXTINCTION: DISTINGUISHING BETWEEN SENSORIMOTOR AND TURNING ASYMMETRIES IN RATS WITH UNILATERAL DAMAGE TO THE SUPERIOR COLLICULUS. T. Barth*, and T. Schallert. Dept. Psychol., Univ. Texas, Austin, TX 78712

A traditional view of the function of the superior colliculus is one of multimodal sensorimotor integration. This view is based in part on the fact that unilateral collicular damage severely impairs orienting to sensory stimuli (e.g., von Frey hairs) presented on the contralateral side of the body. However, in the absence of sensory stimulation, animals with unilateral collicular damage display an ipsilateral postural and turning bias. Some investigators (Cooper et al., J. Comp. Physiol. Psychol., 72:286, 1970; Marshall, Exp. Neurol., 58:203, 1978) have suggested that the orienting deficit, which is the failure to make a head movement in the direction of the sensory stimulus, may be directly related to this ipsilateral movement bias rather than to an impairment of sensorimotor integration. The present study addresses this problem with the use of a sensitive sensorimotor test that is not confounded by stimulus-independent postural and motor asymmetries. This test is a modification of a "tactile extinction" procedure used in the evaluation of sensorimotor symmetry in human patients with parietal or frontal lobe damage.

Small pieces of adhesive paper were applied bilaterally to various parts of the limbs or snout, and latencies to remove the stimuli were recorded. Because head and body movements are not required in this task, assessment of stimulus-directed movement asymmetries could be quantified without the influence of stimulus-independent postural and turning asymmetries. The utility of this test has been demonstrated previously in rats with electrolytic lesions of the substantia nigra. These rats, despite having a strong contralateral motor bias, display an ipsilateral adhesive removal bias similar to that seen in rats with unilateral 6-OH-dopamine induced nigrostriatal damage (Schallert et al., Pharm. Biochem. Behav., 16:455, 1982).

Unilateral superior colliculus lesions produced the expected long-term contralateral deficit in orienting to von Frey hair stimulation and ipsilateral motor biases as determined from a battery of tests of spontaneous movement. However, the "tactile extinction" test revealed that there was no change in sensorimotor behavior. That is, despite severe ipsilateral motor asymmetries and contralateral neglect of von Frey hair stimulation, the latencies to remove the ipsilateral vs contralateral adhesive stimuli were not different from preoperative values. The role of the superior colliculus in sensorimotor integration will be discussed in light of these data. (Supported by NIH grant NS-17274 to T.S.)

273.3 DISSOCIATION OF VISUAL AND ACOUSTIC DEFICITS FOLLOWING UNILATERAL AND BILATERAL LESIONS TO THE SUPERIOR COLLICULUS IN THE BAT PHYLLOSTOMUS HASTATUS. J. Chase,* L. Rubin,* V. Chang,* and G. Ball.* Barnard College, Columbia Univ., New York, NY 10027.

The function of the acoustic inputs to the deep layers of the Superior Colliculus is not well understood. Since deeply lesionned animals fail to turn toward either visual, acoustic, or tactile stimuli, it has been hypothesized that the SC is important not only in visual but also in acoustic orientation, attention and localization. Using the bat P.hastatus which can navigate both visually and acoustically (echolocation), we tested the effect of SC lesions on acoustic orientation. Bilateral lesions significantly diminished the bats' ability to fly through a series of barriers visually (ears-plugged), but did not affect acoustically guided trials (blindfolded). Unilaterally lesionned bats showed persistent circling, but this ceased immediately when bats were blindfolded. The only deficit detectable on both visually and acoustically guided flights was a persistent bias in unilaterally lesionned bats to turn in the ipsiversive direction. We conclude that the SC is not involved in acoustic orientation and localization and suggest that the prime function of the acoustic input to the SC may be to direct the animal's gaze toward acoustically detected stimuli.

273.4 AUDITORY AND SACCADE-RELATED ACTIVITY IN THE SUPERIOR COLLICULUS OF THE MONKEY. Martha F. Jay & David L. Sparks, Dept of Physiol Biophys/Neurosci, Univ of Alabama in Birmingham, B'ham, AL 35294.

As the superior colliculus contains cells responsive to auditory, visual and somatosensory stimuli, it is a potential site for the convergence of the three sensory systems onto a common motor pathway for the generation of saccadic eye movements. To investigate this question, the activity of superior colliculus cells was observed prior to saccades to both auditory and visual targets. Also, the coordinate system in which sound sensitive cells encode target position (head, retinal or motor) was studied by measuring the response of these cells to noise bursts while the orbital position of the eyes was varied.

Rhesus monkeys, implanted with eye coils, were trained to perform delayed saccade tasks in order to receive a liquid reinforcement. The delayed saccade trials provided a temporal separation of sensory and motor activity, recorded extracellularly in the superior colliculus, by requiring the subject to wait 300-700 msec after the onset of a visual or auditory stimulus before making a saccade to that target.

Most visual-motor cells were activated prior to sound-induced saccades but had no sensory response to sounds. Other units could be called "visual/auditory-motor" cells as they showed a short latency increase in firing rate temporally correlated with the onset of either an auditory or visual target along with a second response beginning up to 100 msec prior to a saccade to that target. The magnitude of both sensory responses was dependent on the position of the eye in the orbit. "Auditory-motor" cells responded to auditory targets and discharged prior to sound-induced saccades but were not visually responsive nor were they active prior to visually-induced saccades. Similarly, strictly visual-motor cells that did not participate in saccades to sounds were found. Saccade-related burst cells discharged prior to saccades made to targets of either modality. Generally, the magnitude of this pre-saccadic burst was reduced for sound-induced saccades relative to those to visual targets. The auditory-motor cells were ventral to visual-motor and saccade-related burst cells but were still in a low threshold region for eliciting saccades with stimulation ($\leq$20 μA).

The hypothesis that the superior colliculus represents a site where signals from different sensory systems are translated into a common motor command is supported by the observation that some collicular neurons discharge before saccades to both visual and auditory targets. This convergence is not absolute as the populations of neurons discharging prior to sound- and visually-induced saccades only partially overlap. (Supported by EY01189 & P30 FY03039)

273.5 TECTAL INFLUENCE ON THE CERVICAL SPINAL CORD OF THE CAT: ANATOMICAL DEMONSTRATION OF DIRECT AND INDIRECT PATHWAYS, M.F. Huerta and J.K. Harting*, Dept. Anatomy, Univ. of Wisconsin, Madison, WI 53706.

Anterograde (^{3}H-amino acids) and retrograde (horseradish peroxidase, HRP) transport methods were used to demonstrate direct (tectospinal, TS) and indirect pathways by which the superior colliculus (SC) may influence the cervical spinal cord grey (C_1-C_8).

Regarding the tectospinal tract, anterograde data reveal that TS axons ramify primarily within laminae VI-VIII of the contralateral spinal cord segments C_1-C_5; sparse tracer is also apparent in lamina IX, the location of neck motoneurons. HRP data indicate that 75% of TS cells occupy the intermediate grey layer of the SC; most of the remaining lie in the deep grey. TS cells, like many other tectofugal neurons, occur in clusters. Such clusters are especially prominent within lateral regions, and are suggestive of a modular connectional organization (e.g., Huerta et al., Brain Res., 211:1).

By comparing anterograde and retrograde data, we have found that in addition to the tectospinal tract, several multisynaptic pathways exist over which collicular information may influence motoneurons innervating the neck musculature. Thus, the contralateral supraspinal nucleus of the caudal medulla receives substantial input from the SC; this nucleus projects bilaterally to laminae VIII and IX of C_1-C_5. In addition to the supraspinal nucleus, extensive regions of the reticular formation receive input from the intermediate and deep layers of the SC and project to C_1-C_8. These brainstem zones include the: MRF, Rpo, Rpc, Rgc and Rv (Brodal, '57). In particular, the Rpo-Rpc and the Rpc-Rgc interfaces receive especially dense SC input and contain many cervically projecting neurons. Furthermore, these two brainstem areas also possess connections with structures related to eye movements. Thus, tectal signals to the Rpo-Rpc and Rpc-Rqc interfaces may play a role in the coordination between eye and head movements.

In addition to brainstem structures, our findings also demonstrate several other nuclear zones which both receive SC input and send axons to C_1-C_8. These areas include the: zona incerta, posterior hypothalamic area, nucleus of the posterior commissure and interstitial nucleus of Cajal.

These connectional studies reveal many pathways which account for both physiological and behavioral data which implicate the SC in orientation responses of the head as well as eye-head movement coordination.

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273.6 AFFERENT PROJECTIONS TO THE CENTRUM MEDIANUM IN THE RAT AS DEMONSTRATED BY RETROGRADE LABELING OF HORSERADISH PEROXIDASE. D. S. Yamasaki, G. M. Krauthamer and I. M. Cassidy*. Dept. of Anatomy, UMDNJ - Rutgers Medical School, Piscataway, NJ. 08854.

The afferent projections to the centrum medianum (CM) of the intralaminar thalamus have been studied in the cat (McGuinness, C. M., and Krauthamer, G. M., Br. Res. 184:255, 1980). In this study, afferent projections to homologous regions in the rat were mapped with horseradish peroxidase (HRP). Small electrophoretic injections of HRP (TMB method) were placed into a zone of the medial thalamus which is at the origin of a massive projection to the neostriatum (Lentini, J. F., and Krauthamer, G. M., Soc. Neurosci. Absts., 5:75, 1979).

The majority of labeled neurons were found in the ipsilateral stratum griseum profundum and stratum griseum intermediale of the superior colliculus, ipsilateral substantia nigra pars reticulata, precentral cortex ipsilaterally, periaqueductal gray and reticular formation bilaterally, and zona incerta ipsilaterally. Labeled cells were also seen in the medial, lateral, and superior vestibular nuclei, ipsilateral entopeduncular nucleus, ipsilateral stratum album intermediale of the superior colliculus, and contralateral fastigii and globosus nuclei of the cerebellum.

These results suggest, as in the cat, that CM may play an important role in the integration between the neostriatum and the orientation related responses of the deep tectum and vestibular nuclei.

273.7 CORTICAL CONNECTIONS OF CINGULATE AREA 29d: A LIMBIC SENSORIMOTOR ASSOCIATION CORTEX. Brent A. Vogt and Michael Miller. Dept. of Anatomy, Boston University School of Medicine, Boston, MA 02118.

Although corticocortical connections may play an important role in sensorimotor interactions, little is known about this class of connections in the rat brain. An analysis of the cortical sensory and motor connections of cingulate cortex suggests that area 29 may be involved in visuomotor integration.

Injections of horseradish peroxidase (HRP) into area 29c-d results in retrograde labelling of neurons mainly in layer V of visual areas 18a, 18b and 17 as well as in layers III-V of supplementary motor area 8. Neurons also label in medial limbic structures including cingulate (areas 25 and 24) and parahippocampal cortices (presubiculum, area 27; postsubiculum, area 48; subiculum).

Many of these connections are confirmed by injections of tritiated amino acids (AA) into visual cortex. Following injections of AA into area 17, termination is present in areas 29d, 8, 27 and 48. A similar projection pattern occurs following injections into area 18b, but termination in cingulate cortex is more extensive for it includes all subdivisions of area 29.

There are three reasons for proposing that connections between cingulate and visual areas are reciprocal. First, HRP injections into areas 17 and 18b result in HRP labelling of neurons in layer V of areas 29d and 29b. Second, AA injected into area 29d are anterogradely transported to area 18b and medial area 17. Third, anterograde as well as retrograde transport of HRP occurs in area 18b following an HRP injection into area 29c-d. The connection between cingulate and supplementary motor cortices may also be reciprocal, since injections of AA into area 29d demonstrate termination in area 8, while HRP injections into area 29c-d result in retrograde labelling of neurons in area 8.

Thus, two types of cortical connections may subserve visuomotor integration in the rat brain. First, direct neocortical projections of areas 17 and 18b to area 8, and second, via connections with limbic cortex. Area 29d in particular has reciprocal connections with visual cortex and also projects directly to supplementary motor area 8. Therefore, area 29d may be classified a limbic sensorimotor association cortex.

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274.1 CALORIC-INDUCED AUGMENTATION OF H-REFLEXES IN NORMAL, BUT NOT IN SPINAL CORD INJURED PATIENTS. D.H. York and M. Raffensberger*, Dept. of Physiology, School of Medicine, University of Missouri, Columbia, MO 65212

The ability to definitively test anterior spinal cord motor function in an individual with a spinal cord injury has been a difficult problem. Non-invasive accessibility to descending spinal cord motor tracts in man is limited. The present study was undertaken to test the hypothesis that vestibulospinal tract activation will cause a defined reproducible change in the monosynaptic reflex of the lower limb. The demonstration of such an interaction would allow assessment of anterior cord function and possible prognostic value in predicting recovery of motor function in spinal cord injury.

Eighteen subjects, 8 males 10 females with a mean age of 27.8 ± S.D. 4.6 years were evaluated. All subjects had a negative history regarding ear infections and neurological disease. An H-reflex was recorded by means of a silver chloride surface electrode placed on the medial aspect of the gastrocnemius muscle, after initial cleaning and abrading of the skin resulting in electrode impedence >5 kohms. A reference electrode was placed medial to the achilles tendon and a ground was placed on the gastrocnemius proximal to the recording electrode. Constant current square wave stimulus pulses (7-20 mA, 1.0 msec, 1/10 sec) were delivered to the tibial nerve by a bipolar surface electrode at the popliteal fossa. Current intensity was adjusted to produce an H-reflex amplitude one half maximal which was usually below threshold for the M response. At least four control trials of 5 responses each were averaged in a CA 1000 signal averager (filter 30-3000 Hz, gain 10) before caloric stimulation. Once control trials demonstrated a consistent H-reflex amplitude, a caloric stimulus (irrigation of the external auditory canal with ice water) was delivered to either ear until the individual reported dizzyness and visual nystagmus was observed. An H-reflex trial was then begun, and repeated with 30 sec break between trials. Only those trials which recovered to control levels following caloric stimulation were analyzed statistically. The 9 subjects tested, who returned to control showed a 2.1 + S.D. 0.87 mV (56%) increase in H-reflex amplitude over control following caloric stimulation. Two subjects who demonstrated no dizzyness or nystagmus despite prolonged caloric stimulation, also did not demonstrate any significant alteration in H-reflex amplitude. Three paraplegics who were at least one year post accident and demonstrated clinically complete lesions were also tested. In no case was a change in H-reflex amplitude observed. Thus the integrity of the spinal cord is necessary to demonstrate caloric modulation of H-reflexes. Furthermore, vestibulospinal tract by its anterior position in the spinal cord may offer a method of assessing anterior cord function in a patient with an incomplete lesion, which would offer reinforcement for active rehabilitation management.

274.2 IMPAIRED CONTROL OF OROFACIAL MUSCLE FORCE IN CONGENITAL SPASTICS, S. M. Barlow* (SPON: R. Goldstein). Speech Motor Control Labs, Waisman Center, University of Wisconsin, Madison 53706.

Spasticity has been defined as an episodic hypertonicity that is induced by rapid movement or attempts at such movements (Landau, W., Arch Neurol., 31, 1974); hence, there is substantial potential for interference with the precisely controlled muscle contractions. Clinical neurophysiologists (Rosenfalck, A., and Andreassen, S., J Ne Ne Psych., 43, 1980), investigating select force control variables involved in movement of the limbs, have found that spastic patients manifest control impairments in the generation and maintenance of static isometric force. If the submaximal contraction instability present in limb muscles is also present in the bulbar musculature, it may have serious implications for the motor skills required for the complex movements involved in deglutition and speech production. Given the above considerations, submaximal isometric force control of the labial, mandibular, and lingual motor subsystems was investigated in seven adult humans with congenital spasticity and in normal subjects matched for age. All subjects were asked to produce several levels of steady, submaximal isometric forces at prespecified target levels monitored on a storage oscilloscope. Force signals were digitized via a PDP-12 computer and a quantitative measure of force control instability around the target force level was obtained using a digital algorithm to calculate signal standard deviation.

In contrast to the normals, submaximal isometric force control was significantly impaired in the spastic subjects. There was a strong tendency for the jaw and tongue subsystems to show a greater degree of force control instability than the labial motor subsystem within the ranges studied. In contrast to the lips, the degree of force control impairment was always greater for the lingual and mandibular motor subsystems. It is interesting to note that both the lingual and mandibular subsystems are well endowed with muscle spindles whereas there is an absence of muscle spindle receptors in the labial musculature.

The absolute force control instability profiles were often grossly different between two motor subsystems for a given subject supporting the hypothesis of differential subsystem involvement in patients with upper motor neuron dysfunction. In contrast to normals, some patients with spasticity manifest a paradoxical increase in contraction instability with decreasing force levels. These latter findings have obvious implications for the execution and performance of fine motor skills, e.g., speech production, since the presumed muscle forcing functions are thought to involve submaximal force generation.

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274.3 STIFFNESS COMPARISONS IN NORMAL AND SPASTIC LIMBS OF HEMIPARETIC SUBJECTS. W.A. Lee, A. Boughton* and W.Z. Rymer. Department of Physiology, Northwestern University and the Rehabilitation Institute of Chicago, Chicago, IL 60611

This study tested the hypothesis that stretch reflex gain (estimated by static stiffness) would not differ for the spastic and normal elbows of spastic hemiparetic persons, when intial conditions of joint angle and force were carefully matched across sides. Seventeen volunteer hemiparetic subjects (etiology of cerebrovascular accident or head trauma) were tested in 1 to 5 sessions of a load perturbation task. Prestimulus and perturbation load values were selected on the basis of the strength in each individual's weaker, spastic arm. In each session, 8 to 12 repetitions of each load perturbation (step load 2.5 sec duration, random load order) were applied to each of the arms and the resultant displacement measured. The task was self-paced and rests were provided to minimize fatigue of the spastic limb. Stiffness was measured as the ratio of incremental force to incremental position, measured at steady state (2.0-2.5 sec post-perturbation). Of 9 subjects tested who could perform the task adequately, six demonstrated little (<20%) difference in stiffness between normal (K=4.52 N/cm) and spastic sides (K=4.49 N/cm); two subjects showed greater stiffness on the spastic side (K=4.89 N/cm; normal, K=3.47 N/cm), and one had the spastic limb stiffer on one day but the normal limb stiffer on a second day. Between-limb differences in stiffness were consistent across days for individual subjects, with the one exception noted. Electromyographic data suggest that some differences in stiffness may reflect either co-contraction effects or the occurrence of subtle voluntary reactions.

The results supported the hypothesis that stretch reflex gain is not necessarily augmented in spasticity, as frequently has been presumed. The data emphasize the likelihood that increased resistence to stretch in spastic limb reflects a disturbance in stretch reflex threshold, not in reflex gain.

274.4 MEDULLARY RETICULAR FORMATION: SITE OF STIMULUS-SENSITIVE MYOCLONUS IN RATS. M.H. Van Woert and E. Chung. Dept. Neurology and Pharmacology, Mount Sinai Sch. of Med., New York, N.Y. 10029.

Intragastric administration of p,p'-DDT (100-600 mg/kg) to rodents has been used as an animal model of stimulus-sensitive, arrhythmic, serotonin-responsive myoclonus. We previously reported that lower thoracic spinal cord transection eliminated DDT myoclonus below the lesion, while transection of the upper brain stem did not modify DDT myoclonus, indicating that the myoclonus is generated within the brain stem or cerebellum. To further localize the neuronal origin of myoclonus, we infused p,p'-DDT directly into various brain areas.

Local unilateral infusion of p,p'-DDT (0.5 mg in 5 ul dimethylsulfoxide) into the medullary reticular formation (MRF) (ranging from 8.5 to 10.5 mm posterior to bregma) produced generalized arrhythmic, stimulus-sensitive myoclonus in rats similar to that obtained following intragastric injections of p,p'-DDT. This level of the MRF includes nucleus gigantocellularis, nucleus parvocellularis, nucleus paramedianus and the anterior portion of the lateral reticular nucleus. Intraperitoneal injection of L-5-hydroxytryptophan (100 mg/kg) plus chlorimipramine (10 mg/kg) reduced the myoclonus produced by local infusion of p,p'-DDT into MRF, as well as its intragastric administration. Injection of vehicle alone in these regions had no effect. In addition, injection of p,p'-DDT into the pontine, mesencephalic and most caudal part (below 10.5 mm posterior to bregma) of MRF did not produce any neurological signs. Furthermore, injection of DDT into other brain areas, such as caudate, hippocampus and substantia nigra, also did not produce myoclonus.

p,p'-DDT is known to cause repetitive neuronal responses to a single stimulus. p,p'-DDT myoclonus appears to be due to stimulus-sensitive hyperexcitability of neurons in the MRF, resulting in an enhancement of the spino-reticular-spinal reflex. (Supported by USPHS grants NS 12341 and NS 17258).

274.5 MOTONEURON INPUT RESISTANCE AND F-I RESPONSES IN IDPN NEUROPATHY. Denise A. Delio* and Herbert E. Lowndes, Dept. Pharmacology, N.J. Medical School, Newark, N.J., 07103.

β-β'-iminodipropionitrile (IDPN) induces giant axon formations (GAF) in proximal axonal regions of anterior horn cells. Previous studies in cats exposed to IDPN for 35 days demonstrated that GAF alter function in motoneurons (MN), characterized by repetitive firing upon single stimulation and abnormal action potential formation. These data suggested that MNs are hyperexcitable. The present study further tested MN excitability by direct current injection and determination of input resistance (Rin). Intracellular recordings from MNs in cats treated with IDPN, 50 mg/kg i.p., once weekly for 35 days revealed higher thresholds for firing (controls=9.1 nA, IDPN=14 nA) but greater firing frequencies (16.5 vs 20.1 Hz) to injected current. While all MNs exhibited a primary range of firing frequencies only 37% fired in the secondary range. The transition from primary to secondary ranges occurred at higher current strengths (controls=16.9 nA, IDPN=24 nA), accompanied by increased firing frequencies (39.4 vs 46 Hz). The slopes of primary (control=3.1 Hz/nA, IDPN=2.57 Hz/nA) and secondary (9.19 vs 3.3 Hz/nA) ranges were reduced from those in control animals. Both maximum current strength (controls=20 nA, IDPN=30.2 nA) and firing frequencies (66 vs 69 Hz) were increased. Input resistances fell into three subpopulations: low (0.53 MΩ, n=9), high (6.1 MΩ, n=11) and normal (1.69 MΩ, n=6). Corresponding values in untreated cats were 2.16 MΩ, n=14. Preliminary data from animals treated for 7 and 14 days indicate similar input resistances. These results demonstrate that MNs of IDPN-treated cats have a higher threshold for initiation of action potentials, but once threshold is achieved, the cells discharge rapidly and continue to respond to higher than normal amounts of current. Afterhyperpolarization, which limits the firing frequencies of MNs is a major determinant of primary and secondary firing ranges. Since afterhyperpolarization is reduced in MNs of treated animals, this may partially explain the enhanced firing frequencies observed and the relative inability to shift from primary to secondary ranges. Concomitant chromatolytic changes may occur as a consequence of the GAF and the different sizes of GAF may underlie the heterogenous subpopulations of input resistances as well as influence the ability of the MN to respond normally to injected current.

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274.6 FITTS' LAW AND MOVEMENT TIME OF PARKINSONIAN PATIENTS. J. N. Sanes, Lab. of Neurophysiol., NIMH, Bethesda, MD 20205

Patients with Parkinson's Disease (PD) exhibit inaccuracy of movement when movements are completed in less than one reaction time (<200 ms). The present study evaluates quantitative relationships between movement time (MT) and movement accuracy using the method of Fitts (J. exp. Psychol., 1954, 47, 381), who showed that systematic changes in movement and target size resulted in movements that are scaled in time provided accuracy is held constant. Thus, movements of equal size but different difficulty (in relation to target size) are performed at different rates, with the movement directed between larger targets being performed faster. Since PD patients fail to perform accurate rapid movements, it might be expected that changes in a movement's index of difficulty would cause greater increments in MT for PD patients than for normal subjects.

Patients with PD and age matched controls held an electrical stylus that was alternately touched to two targets in a series of 20 rapid movements. Target width was 1-4 mm on one set of target arrays and from 0.5-4.0 cm on a second set of target arrays. The distance between targets was 2-8 mm or 4-32 cm for the respective target arrays. Movements by normal subjects were required to be within a 95% accuracy criterion, whereas PD patients were encouraged to move as accurately and as rapidly as possible.

Variation of movement amplitude and target width altered MT of both normals and patients with PD. Normals and PD patients had different performance on the smallest targets (widths of 1-4 mm, amplitudes of 2-8 mm) such that PD patients were slower and less accurate than normals for all combinations of target size and distance moved. The performance of PD patients on the larger target arrays was slower and less accurate than normals but there was also a steeper rate of change in MT as movement size increased for the various target widths. The greater slope of MT/cm for PD patients for all target widths was related to substantial increases in MTs for the largest movements. Patients with PD also showed slower MTs when the target was small.

These findings demonstrate that changes in movement size and target width will modify MT in PD patients different from that of normal subjects. The primary deficit in MT for PD patients was the failure to accurately execute large amplitude movements and movements to small targets. Thus, as the index of difficulty increases (independent of the source of the increase) patients with PD move slower than normals. Therefore, equations relating MT, accuracy and movement amplitude differ between normals and patients with PD. Since emphasis was given to rapidity, as well as accuracy, of movement it may be expected that patients with PD would perform reasonably well in tasks of this type if they are given ample time to complete a movement.

274.7 DECREASED CATALEPTIC RESPONSE TO HALOPERIDOL IN TWO DYSTONIC MUTANTS: THE RAT MUTANT DYSTONIC (dt) AND THE MOUSE MUTANT DYSTONIA MUSCULORUM (dt^J). Tina Williams McKeon and Joan F. Lorden. Departments of Anatomy and Psychology, University of Alabama in Birmingham, Birmingham, AL 35294.

A new model of inherited movement disorder in Sprague-Dawley rats has been discovered and characterized as an autosomal recessive mutation. Behavioral observations indicate that the rat mutants (named dystonic, dt) are indistinguishable in the open field from normal littermates until 10 to 11 days of age when the mutants begin to exhibit a stiff paddling gait with frequent falling to the side, excessive pivoting or circling, and alternating torticollis. Prior to that age, mutants and normal littermates are equally capable of performing advanced motor tests such as climbing a wire mesh incline. Comparable weight gain also indicates that the rat mutants are able to compete with normal littermates for nutrition through 16 days of age.

Preliminary anatomical observations indicate that the pathogenesis of dystonia in the rat is distinctly different from dystonia musculorum in the mouse (dt^J and dt^{A16}). Anatomical changes in the central and peripheral sensory pathways reported in the mouse mutant by Duchen et al. (Brain 87: 367-378, 1964) are not revealed in rat mutants less than 30 days old using routine light microscopy. Also pathological changes in the red nucleus and striatum of the mouse mutant reported by Messer and Strominger (Neuroscience 5: 543-549, 1980) are not apparent in the rat mutant 19-23 days old.

Since previously reported neurochemical data from the dystonic rat (Neuroscience Abstracts 7: 783, 1981) indicate an increased number of striatal dopamine (DA) binding sites but normal DA levels, haloperidol-induced catalepsy was used to assess the functional integrity of DA receptors in both rat and mouse models of dystonia. The mice received 5 mg/kg of haloperidol while the rats received 2.5 mg/kg. Latency to resume a normal posture following placement of the forelimbs on an elevated bar was used as a measure of catalepsy. Catalepsy was not elicited in 19, 29, or 49 day old dystonic mice, while all normal littermates showed positive cataleptic responses. Dystonic rats of 8 and 10 days of age were indistinguishable from normal littermates. At 12 days, however, dystonic rats showed a delayed response to haloperidol, suggesting a decreased sensitivity to the drug.

These preliminary neurochemical and pharmacological findings suggest that the dystonic rat may have an alteration in striatal DA receptors or in a subsequent point in the pathway by which catalepsy is mediated. A similar defect may also be present in the mouse model. (Supported by NINCDS grant NS18062).

274.8 THE EFFECT OF TRIETHYLTIN INTOXICATION ON PROXIMAL AND DISTAL PERIPHERAL MOTOR AND SENSORY NERVE CONDUCTION VELOCITIES. E. Aizenman*, E.F. Stanley, G.G. Bierkamper*. (SPON: C. Andrew) Laboratory of Neuromuscular Toxicology, and the Department of Neurology, The Johns Hopkins University, Baltimore, MD 21205

Triethyltin (TET) induces neuropathic changes in both human and experimental animals. This compound has been shown to cause extensive myelin splitting in the central nervous system and to a lesser extent in peripheral nerves. Damage to myelin is known to impair action potential conduction velocity. In order to evaluate the effect of TET on peripheral nerves, we have examined nerve conduction velocity (NCV) in proximal and distal segments of motor and sensory nerves in rats.

Adult male Long Evans rats (300-380g) were exposed to TET bromide (ICN) in their drinking water (30 mg/l) ad libitum for 3 weeks; age-matched and water restricted-age and weight-matched controls were also tested. Motor and sensory NCV as well as H-reflex latencies from the sciatic nerve were assessed by a method previously described by Stanley (Exp Neurol 71:487, 1981). A variant of this method was used to examine conduction in the dorsal and ventral roots. Monopolar needle electrodes were inserted in the interlaminar space at L1/L2 and L4/L5 while recording from the plantar muscles of the foot. Monosynaptic H-reflexes or motor responses were evoked by varying the stimulus intensity and were used to measure proximal motor and sensory NCV and the central delay of the reflex pathway.

Physiological findings after 3 weeks treatment were:

		TET	Age Controls
H-Reflex	Central delay (L1) ms	1.6 ± 0.1	1.6 ± 0.1
Motor NCV	proximal (L1-L4) m/s	50.5 ± 3.1	56.3 ± 5.4
	distal (SN-ankle) m/s	39.8 ± 2.9	43.9 ± 1.4
Sensory NCV	proximal (L1-L4) m/s	20.7 ± 2.5**	45.1 ± 4.0
	distal (SN-ankle) m/s	42.2 ± 3.7	45.7 ± 2.0

**p< 0.01

No differences were found between age-matched and age/weight-matched controls. SN = Sciatic notch.

Our results show a reduction of NCV in the segment of the H-reflex arc involving the dorsal roots whereas the NCV of the distal sensory fibers, ventral roots, and the distal motor fibers are normal. The proximal branch of sensory fibers may therefore show a selective vulnerability to TET.

This work was supported by a grant from NIEHS, ES 02645.

274.9 ELECTROCONVULSIVE SHOCK: MOTOR PARALYSIS FOLLOWING SUB-CONVULSIVE STIMULI. L. Isaac and C. Advokat. Dept. Pharmacol., University of Illinois College of Medicine, Chicago, IL 60612.

During the course of an investigation to determine the electroconvulsive threshold of rats we observed that some animals appeared paralyzed, that is, they did not use their limbs in locomotion but did withdraw the limb to a painful stimulus. Because the paralysis was reversible, we thought it would be of value to investigate the relationship between electroconvulsive treatments and this dysfunction.

The electrical current (60 Hz, A.C., 0.1 sec) was delivered through ear-clip electrodes to 225 g male rats. Electroconvulsive treatments consisted of a single convulsive stimulus (65 mA) and/or an ascending series of electrical stimuli (40, 45, 50, 55, and 60 mA delivered at 10-15 min intervals). If any stimulus in the ascending series evoked a tonic extensor response, treatment at that time was discontinued.

Animals that were convulsed twice a day (four hours apart) with 65 mA exhibited no functional impairment. On the other hand, almost half of those treated with the ascending series of electrical stimuli, at least once a day, lost voluntary control of their limbs. Although forelimb paresis did occur, hindlimb dysfunction was more commonly seen. The first signs of paralysis were usually observed following two ascending series of stimuli. The severity and rate of recovery of the dysfunction was quantified by the inclined plane method of Rivlin et al., (J. Neurosurg. 47:577, 1977). We found that unimpaired rats could maintain themselves on the inclined plane at an angle of 60° whereas, twenty-four hours after paralysis, the injured animals could maintain themselves at an angle of only 30°. At this time, we observed that rectal temperature was normal; and that tail-flick latency to a thermal stimulus was normal and paw-pinch withdrawal was present indicating that spinal reflexes were intact. Although 20% of the paralytic animals died, the remaining animals recovered within 7 days (maintained themselves on the incline at an angle of at least 60°).

To our knowledge, these findings are the first to describe a motor impairment in rats that have received electroconvulsive treatments. In conclusion, (1) the fact that the limb musculature is functional (tail-flick latency and paw-pinch withdrawal) suggests that the lesion resides within the central nervous system and (2) the paralysis is the result of the cumulative consequences of the ascending series of electrical stimuli rather than the total stimulating current administered at one time.

274.10 SPINAL ROOT PROTEINS IN MOTONEURON DISEASE. T.O. Brock, III and D.L. McIlwain. Neurobiology Program and Department of Physiology, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514.

Total protein extracts from the lumbar and cervical spinal roots of 4 patients who had amyotrophic lateral sclerosis (ALS) and 2 who had Werdnig-Hoffmann disease (WHD) were compared with similar extracts from 5 normal adults and 1 infant, using two-dimensional polyacrylamide gel electrophoresis. Two classes of abnormalities were noted in the proteins from the diseased spinal roots. The first was the presence of a group of proteins ranging in molecular weight from 39-50K with isoelectric points of 5.0 to 5.8. This group of proteins, containing up to approximately 30 spots on silver-stained gels loaded with 15 ug protein, was especially prominent in extracts of the WHD ventral roots. Some of these proteins were also present in lesser amounts in the dorsal roots of 1 of the 2 WHD patients and in all 4 ALS dorsal and ventral roots. The proteins were found in both lumbar and cervical spinal roots in each disease. They were either absent or detectable in only trace amounts in normal infant or adult dorsal and ventral roots. These proteins were also particularly prominent in soluble protein fractions from normal human ventral gray matter. Their abundance in WHD ventral roots and in normal central nervous tissue strongly suggests that these proteins originate from the glial bundles which are well-known morphological abnormalities of WHD ventral roots (Chou et al., Acta Neuropathol.41: 45-54, 1978). Recently, Ghatak et al.(Ann. Neurol. 11: 203-206, 1982) has detected the presence of small numbers of glial bundles within the ventral, but not the dorsal roots of a patient with ALS.

The second type of abnormality observed in the diseased spinal roots involved quantitative alterations in some of their cytoskeletal proteins. Tubulin was substantially reduced in both the dorsal and ventral roots of the 2 WHD patients. The 68K neurofilament polypeptide was reduced in WHD and ALS ventral roots. Control experiments with bovine spinal roots suggest that post-mortem degradation alone cannot account for these decreases (Brock and McIlwain, Neurosci. Abstracts 7: 907, 1981).

In summary, these findings indicate that there are multiple biochemical abnormalities in dorsal, as well as ventral roots in these two motoneuron diseases.

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274.11 THERAPEUTIC EFFECTS OF FES IN THE CORRECTION OF FOOT-DROP IN CEREBRAL PALSY. R.R. Riso, K. Sutin*, J.T. Makley* and P.E. Crago. Rehabilitation Engineering Ctr., Depts. of Orthopaedics and Biomed. Engr., Case Western Reserve Univ., Cleveland, OH. 44106

We have sought to evaluate the therapeutic effects of Functional Electrical Stimulation (FES) on abnormal neuromuscular control. As a model we are studying the effects of stimulation of the peroneal nerve on the walking function of cerebral palsy children having either foot-drop or spastic equinus gait. Instrumented gait analysis is used to document changes in gait that are induced by the FES, with an emphasis on detecting any features that remain altered after the stimulation has ceased. As the subject walks down a conductive walkway we measure bilaterally: foot placement patterns using footswitches; EMG's of the ankle flexor and extensor muscles obtained through surface electrodes, and flexion-extension of the ankle of the treated leg using an electro-goniometer. Initially, each patient was evaluated on two or three occasions to obtain baseline performance. At all subsequent evaluations gait parameters were measured before, during and immediately after a 20 min period of FES in order to assess any effects from "short term" stimulation. After about 5 test sessions each patient took a stimulator home for daily FES and returned to the lab at 2 weeks and then roughly monthly intervals for evaluation of "long term" effects. For 8 patients who have been studied including 4 hemiplegic and 2 diplegic children with CP: and 2 hemiplegic children who sustained brain damage in later childhood, repeated testing for "short term" effects of FES revealed that the dorsiflexion and eversion of the foot obtained when the stimulator was in use has never been sustained once the stimulator was turned off, and the patients immediately reverted to their abnormal gait. Long term use of the FES (6 months to > 1 yr) has also failed to produce automated dorsiflexion during gait that would persist beyond the period of stimulation in most cases with two notable exceptions: For one hemiplegic subject, who had a dynamic equinus deformity during gait, several weeks of FES converted his toe-first stance into a heel strike-first stance. This positive effect was "carried-over" between applications of the FES, but was lost when the stimulation was discontinued completely. It was then demonstrated that the same beneficial effects could be reinstated and maintained by giving the same patient 20 min of FES, 5 days/week while he sat in a chair and used an exercise stimulator. Another child who has spastic diplegia previously walked exclusively on his toes but was able to achieve heel contact during walking after he had received daily FES treatments for several months. (Supported by NIHR grant #G001005815 to the R.E.C. and through collaboration with the Motion Studies Lab of the Cleveland V.A. Med. Ctr.).

274.12 COORDINATED TWO MODE GRASP IN THE QUADRIPLEGIC INITIATED BY FUNCTIONAL NEUROMUSCULAR STIMULATION. P.H. Peckham*, G.B.Thrope*, A.A. Freehafer*, and M.W. Keith*, (SPON: F.T. Hambrecht). Rehabilitation Engineering Center, Departments of Orthopaedics and Biomedical Engineering, Case Western Reserve University, Metropolitan General/Highland View Hospital, Cleveland, Ohio 44106.

Functional Neuromuscular Stimulation (FNS) is a means of providing controlled hand function in cases of high level spinal cord injury (Peckham, P.H., et al., Ann. Biomed. Engr., 8:369, 1980). Versatile grasping is provided when the individual is able to control both lateral prehension (key grip) and palmar prehension (three-jaw chuck grip).

Quadriplegic subjects with a C5 or C6 level or injury are implanted with chronically indwelling percutaneous electrodes. Generally eight or nine muscles are implanted, including the finger flexors (Flexor digitorum superficialis and profundus), finger and thumb extensors (Extensor digitorum communis and Extensor pollicus longus), and thumb intrinsics (Abductor pollicus brevis, Flexor pollicus brevis, and Adductor pollicus). Control of the contractile strength of each muscle is provided through modulation of the stimulus pulse width and interpulse interval, regulating muscle force by recruitment and temporal summation respectively.

The two grasping modes are provided with open loop control. A single proportional command signal regulates the activity of all of the muscles, with coordination of the movement provided by fixed control algorithms. Use of a single patient generated command is necessary to simplify the user's control task. For lateral prehension, the FDP, AdP (or FPB) and EPL are activated, with co-contraction of the latter two muscles providing position control of the thumb. For palmar prehension, the proper position of the thumb opposite to the index and long finger tips is achieved by co-contraction of AbP and EPL, with finger position and force controlled by the FDS and EDC. These grasping modes are provided without use of an external orthosis on the fingers or thumb.

The coordination algorithm for each subject is determined in laboratory studies using a microcomputer controlled multichannel stimulation system. This system enables us to easily modify stimulus parameters to vary the level of muscle recruitment and coordination which will provide the desired grasp. Proper parameters can then be programmed directly from the laboratory computer into a portable stimulation system for patient usage. Ten outpatient subjects are presently using these systems for daily activities such as grooming, eating, and writing.

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274.13 FEEDBACK CONTROL OF ELECTRICALLY STIMULATED MUSCLE, P.E. Crago, G.F. Wilhere*, H.J. Chizeck*, D.M. Saito*, Applied Neural Control Lab., Case Western Reserve Univ., Cleveland, Oh. 44106

Functional neuromuscular stimulation has been shown to be a feasible method of providing function in paralyzed extremities. At present, all clinically applied orthoses are open loop systems, and their performance is limited by the nonlinear time varying relationship between the stimulus parameters and the muscle force or position. Closed loop systems are being designed to improve performance by providing linear, repeatable input-output properties.

In the present study, the stimulated muscle is analyzed as a sampled data system with the sampling period equal to the stimulus interpulse interval (IPI). The difference between the desired contraction and the actual contraction (force or position) is the input to a digital controller. The output of the controller is used to modulate the stimulus parameters. The electrical stimulator is modeled as a sampler whose output is a train of impulses. The area of each impulse is proportional to the magnitude of the modulating signal. The muscle is modeled as a two pole low pass filter with a small pure delay. The nonlinear relationship between stimulus area and the fraction of recruited muscle fibers is modeled as a static nonlinearity between the sampler and the muscle. The output of the muscle is fed back to the controller to complete the system.

A digital controller has been designed to cancel the muscle poles and time delay, and substitute a general second order system. The parameters of the controller are then chosen based on the desired closed loop response properties in the time domain. Controller parameters are chosen to give adequate response properties over a wide range of recruitment gains. Parameters were found that allowed a 10:1 range of recruitment gains without exceeding an overshoot of 20% and a time to peak output of 1.4 seconds in response to a step input. Simulation studies have shown that as recruitment gain varies over a range from one to ten, the overshoot varies from 5% to 20% and the time to peak varies from 1.4 to 0.15 s respectively.

The controller is being evaluated experimentally with isometric cat soleus and plantaris muscles. An IPI is chosen that gives ripple in the output force that is 10% of the peak force and muscle pole locations and recruitment gain are estimated. The responses to step and ramp inputs are measured and compared to responses obtained under open loop conditions. Results indicate that performance is close to that predicted by the simulation tests, and is not unduly sensitive to changes in muscle properties.

This work is funded under contract N01-NS-0-2330 from the Neural Prosthesis Program of NIH-NINCDS.

274.14 ELECTRICAL CHARACTERISTICS OF PLATINUM-IRIDIUM STIMULATING ELECTRODES IN VIVO. D.B.McCreery, W.F.Agnew and L.A.Bullara*. Neurological Research Laboratory, Huntington Medical Research Institutes, Pasadena, Ca.

Platinum-Iridium microelectrodes having low surface roughness were implanted for 3 weeks in the sensory-motor cortex of adult cats. The electrodes were modeled electrically as a simple resistance (access resistance) in series with a parallel network consisting of voltage-dependent incremental capacitance (Cp) and resistance (Rp). When tested with charge-balanced controlled-current pulses, the access resistance was found to be virtually independent of the stimulus current over the range of 5-30 μamps (50-300 $\mu C/cm^2 \cdot ph$). In contrast, Rp was inversely proportional to the stimulus current. When measured at low polarization voltage (close to the start of the stimulus pulse) Cp was also nearly independent of stimulus current. However, at stimulus currents above 10 μamps, Cp was a strong function of the electrode polarization voltage. These findings confirm that access resistance and the parallel elements Cp and Rp reflect distinctly different electrochemical and electrical processes; most probably Cp is the capacitance of the double layer at the metal-tissue interface and Rp is the faradaic resistance of the oxidation-reduction reactions mediating charge injection across the interface.

During prolonged (24 hour) stimulation, all three circuit elements varied considerably, often in episodes lasting for several hours. Specifically, access resistance and Rp showed a positive covariance, but access resistance and Cp varied inversely. Also, Cp was lower for implanted electrodes than for electrodes tested in vitro.

We conclude that Rp and Cp characterize the double layer and are relatively pure indices of the condition and effective surface area of the interface while the access resistance characterizes the surrounding tissue and extracellular fluid. We also conclude that the physical and perhaps chemical environment of the cortex into which the electrodes are inserted can strongly influence the functional surface area factor, at least for electrodes having low surface roughness. Access resistance appears to reflect only that part of the surrounding milieu very close to the stimulating electrode.

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274.15 HISTOPATHOLOGICAL CHANGES ACCOMPANYING ELECTRICAL STIMULATION WITH INTRACORTICAL MICROELECTRODES. W.F.Agnew, T.G.H.Yuen*, D.McCreery, L.A.Bullara* and D.Jacques. Neurological Research Laboratory, Huntington Medical Research Institutes, Pasadena, Ca.

Intracortical stimulations of the sensorimotor cortex of the cat using arrays of Pt-20%IR microelectrodes have been conducted in 28 cats. The arrays contained two or three microelectrodes with beveled tip surface areas of approximately $20x10^{-6} cm^2$. Stimulations utilizing charge-balanced, rectangular waveforms were monitored by computer during 24-hour continuous stimulations using charge densities of 50 to 400 $\mu C/cm^2 \cdot ph$. Immediately following cessation of stimulation the animals were perfused via the aorta with 10% buffered formalin (light microscopy) or 3% glutaraldehyde in 0.1 M phosphate buffer (electron microscopy) using a positive pressure pump. Following perfusion, the electrode arrays were removed, cleaned of debris and the beveled tips examined by scanning electron microscopy (SEM). Results to date indicate that charge densities up to 100 $\mu C/cm^2 \cdot ph$ resulted in minimal neural damage whereas 400 $\mu C/cm^2 \cdot ph$ resulted in severe neural damage following 25 hour stimulations. A striking radial symmetry of neural damage emanated from the electrode tip following stimulation with higher charge densities (>200 $\mu C/cm^2 \cdot ph$) indicating that the uninsulated tip behaved as a point source. The pattern of tissue damage, however, demonstrated a non-linear relationship between neural damage and current density surrounding the electrode tip. A prominent feature of severely damaged electrode sites (400 $\mu C/cm^2 \cdot ph$) was the presence of multi-loculated cavitations 0.4 - 3 μm in diameter, suggesting that gas formation may be a major contributor to neural damage at high charge densities. When charge injection is expressed on the basis of electrode surface area, neural damage thresholds appear to be considerably higher for intracortical microelectrode stimulations compared to those resulting from pial surface stimulations with disc electrodes (Yuen et al., Neurosurgery 9; 292, 1981). SEM micrographs revealed no evidence of corrosion of the electrode tip during the 24-hour stimulations.

274.16 THE EFFECTS OF DENTATE AND INTERPOSED NUCLEI STIMULATION ON VOLUNTARY MOVEMENTS IN SPASTIC PRIMATES. E.G. Hames,* A.B. Schwartz,* T.J. Ebner and J.R. Bloedel. (SPON: F. Torres). Depts. of Neurosurgery & Physiology, Univ. of MN, Minneapolis, 55455.

Previous studies from our laboratory demonstrated that stimulation of the cerebellar surface with appropriate stimulus parameters dramatically improves the abnormal properties of the stretch reflex in spastic primates. Experiments were designed to determine if comparable improvements in abnormal movements performed by spastic monkeys could be produced by stimulation of the cerebellar nuclei. Two Rhesus monkeys were trained to perform a voluntary task with either the right or left hand. This task consisted of moving a manipulandum from a starting position to one of three targets and back again to the starting position. The trajectories of the movement, the velocity profiles as well as the associated EMG activity of the biceps and triceps were plotted and analyzed in groups of ten trials each. After a consistent level of performance was achieved, each animal underwent a unilateral decortication of the left primary motor (area 4) and premotor cortex (area 6). Comparison of the voluntary movements of the right (affected) and left (unaffected) limbs several months after surgery revealed differences in the motor task performed with each hand. A prolongation of the time to complete the entire sequence with increased time spent at the target zone was observed in the movements performed with the limb contralateral to the cortical lesion. The EMG activity of the affected limb exhibited extensive co-contraction of the biceps and triceps. In comparable movements of the unaffected left arm, the EMG activity was reciprocal. Bipolar stimulating electrodes were placed stereotaxically in the right interposed and dentate nuclei. The effects of intermittent dentate and interposed stimulation applied at different phases of the movement were studied. Brief trains of dentate stimuli timed to occur during a specific component of the movement resulted in consistent deviations in the movement trajectory when compared to controls, but did not affect the ability of the animal to reach the target zones. When the stimuli were timed to occur just prior to the start of the movement the animal consistently missed the target zone but the form of the movement trajectory was not modified. These modifications occurred only in the movements made with the right arm and were not accompanied by changes in the EMG activity of either the biceps or triceps muscle. These data show that although dentate stimuli can affect voluntary movements in spastic monkeys, the effects are highly dependent on the time at which the stimuli are applied during the movement. This work was supported by NIH Contract NO1-NS 0-2338.

275.1 THE RESPONSE PROPERTIES OF DSCT CELLS TO PERIODIC MECHANICAL CUTANEOUS STIMULI. D.C. Tam*, T.J. Ebner, and J.R. Bloedel. (SPON: G.W. King). Depts. of Neurosurgery & Physiology, University of Minnesota, Minneapolis, 55455.

This series of experiments examined how neurons of the dorsal spinocerebellar tract (DSCT) encode different features of natural exteroceptive stimuli. In cats anesthetized with alpha chloralose DSCT neurons recorded extracellularly in Clarke's column were identified by their antidromic activation from the ipsilateral cerebellar inferior peduncle. Cells included in this study were those responding to exteroceptive stimuli applied to the ipsilateral hindfoot with a feedback controlled Ling vibrator. Once isolated the responses of these cells to a wide variety of stimulus properties were examined. These included variations in stimulus frequencies (1 Hz to 30 Hz), amplitudes of indentation (0.2 mm to 2 mm), areas of the stimulus probe (5 mm^2 to 200 mm^2) and waveforms. Cycle histograms constructed from the discriminated unitary activity of DSCT cells were examined to determine how these peripheral stimuli may be encoded. They were highly modulated by all types of periodic stimuli used: square waves, sine waves, and triangle waves. These histograms were examined to determine if features of the peripheral stimuli were encoded in either the modulation of the impulse firing or the mean firing rate. Regardless of the differences in the responses evoked by stimuli of different waveforms, the most striking and consistent finding was that the number of spikes per stimulus cycle was inversely proportional to the stimulus frequency independent of the waveform used. The mean firing rate (5-30 Hz) remained relatively constant at stimulus frequencies up to 10 Hz in most DSCT neurons and to higher frequencies in a few cells. Changes in amplitude, wave form, area of stimulus probe or part of the receptive field stimulated did not modify this relationship, even when the temporal firing patterns within the stimulus cycle were modified. These results suggest that DSCT neurons encode the phasic properties of a large variety of stimuli while maintaining a relatively constant mean firing rate. This work was supported by NIH Grants #5R01-NS 13002 and #2R01-NS 09447-10.

275.2 DORSAL ROOT RHYTHMIC DISCHARGES INDUCED BY 4-AMINOPYRIDINE. R. Dubuc, G. Blanchette* and S. Rossignol. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Studies in our laboratory have demonstrated that i.v. injections of 4-aminopyridine (4-AP, 5-20 mg/kg) produced rhythmic discharges (3-7 Hz) in muscle nerves of all four limbs in decerebrate and paralyzed cats. These rhythmic discharges persisted after spinalisation at the first cervical level. It was concluded that 4-AP activated, at different levels of the spinal cord, oscillatory mechanisms which could interact with one another (Rossignol, S. et al., Neuroscience Abstracts, 7, 1981, #280.3). Recently, we have investigated the possibility that the rhythmic discharges seen in peripheral nerves after injection of 4-AP might, at least in part, be due to antidromically conducted discharges in the primary afferents.

Cats were decerebrated at the precollicullar level and the lumbar enlargement was exposed by an extensive laminectomy. Dorsal roots, bathed in paraffin oil maintained at 37°C, were mounted on bipolar Ag-AgCl recording wires. Rhythmic discharges superimposed on large dorsal root potentials (DRPs) were observed following 4-AP injection. After sectioning the dorsal roots, both the discharges and the DRPs persisted proximally whilst they disappeared in the distal segment. Activity in the dorsal roots was bilaterally synchronous and, moreover, the autospectrograms indicated identical frequency components in both. Ventral root discharges were also recorded and found to be cross-correlated to those of the dorsal roots. Rhythmical and bilaterally synchronous discharges were also observed in cutaneous nerves.

In other experiments, rats were chronically spinalized (Th10) in order to produce degeneration of the terminals of descending axons. After a period of 30 days, the rats were anaesthetized with urethane (250 mg/kg) and paralyzed. It was shown that, as in the cat, 4-AP induced bilaterally synchronous rhythmic discharges in muscle and cutaneous hindlimb nerves.

In conclusion, it appears that the centrifugal, bilaterally synchronous discharges observed in the dorsal roots implicate mechanisms which tightly couple primary afferent terminals on both sides and, furthermore, that these mechanisms are inherent properties of the spinal cord which do not require the presence of descending pathways. (This work was supported by a Group grant of the Canadian MRC. R.D. was supported by a studentship of the Québec F.C.A.C.).

275.3 PONS IS REQUIRED FOR MEDULLARY INHIBITION OF MUSCLE TONE. J. M. SIEGEL AND R. NIENHUIS*. V.A. Med. Ctr., Sepulveda, CA 91343, and Dept. of Psychiatry, UCLA, Sch. of Med. Los Angeles, CA 90024.

Magoun and Rhines first demonstrated that stimulation of the medullary reticular formation abolishes tonic muscle activity in the decerebrate cat. It was hypothesized that this effect is mediated by descending polysynaptic pathways from the medulla which produce inhibition of spinal motoneurons. Subsequently, Jouvet and his colleagues discovered that a similar suppression of muscle tone occurs naturally in REM sleep. Lesions placed in a circumscribed region in the dorsal pons eliminate this suppression of REM sleep motor tone. It has been hypothesized that these pontine regions act by projecting caudally to excite the medullary inhibitory area. We have tested these hypotheses by directly stimulating the medullary inhibitory area after transecting the brainstem between the pons and medulla. We found that the inhibitory effect of medullary stimulation on muscle tone is lost after disconnection of the pontine regions.

Surgical procedures prior to brainstem transection were carried out under Halothane anesthesia. The medial cerebellum was aspirated to expose the floor of the fourth ventricle. Blood pressure, percent CO_2, and core temperature were monitored and regulated as required. Two cats were subjected to intercollicular decerebration. Medullary stimulation (500 msec trains of 0.1 msec pulses at 60 Hz) was then applied and produced the previously reported inhibition. A caudal pontine transection, placed just rostral to the abducens nucleus, was then performed. Nuchal muscle tone was comparable to that seen after the first transection. However, medullary stimulation now produced only muscle excitation. Two cats had only the transection at the caudal pontine level performed. No inhibition was produced by medullary stimulation in these animals. An additional two cats had caudal pontine transections performed and were then maintained for 10 and 28 days, at which point medullary stimulations were performed. No inhibition was produced. Unilateral or bilateral excitation of the nuchal musculature resulted from stimulation.

These findings indicate that pontine mechanisms are critically involved in the medullary inhibitory effect. The pontine regions may act directly on motor neurons and form the final common path for medullary inhibition, or may gate descending medullary inhibition at the spinal level.

275.4 L-DOPA INDUCED RHYTHMICAL DISCHARGES IN FLEXOR AND EXTENSOR ANKLE FILAMENTS OF CHRONIC SPINALIZED CATS. L. Baker*, S.H. Chandler, and L.J. Goldberg (SPON: D. Junge). Dept. of Kinesiology and Brain Research Institute, UCLA, L.A., CA 90024; and Dept. of Physical Therapy, USC, Downey, CA 90242.

Recently, fictive locomotion induced by L-DOPA in acutely spinalized cats and treadmill locomotion in mesencephalic locomotor preparations have been used to study the neuronal networks responsible for locomotion. The present study was undertaken in order to compare the ability of these neuronal networks to produce locomotor-like rhythms in normal adult cats acutely spinalized, with cats which have had the spinal cord isolated from higher centers at an early stage of development.

Cats which had sustained spinal transection (T13) at two weeks of age were acutely decerebrated at age six months and 100mg/kg Nialamide and 100mg/kg of L-DOPA were administered i.v. Recordings were made from small nerve branches of identified ankle flexor and extensor muscles concomitant with intracellular recordings of motoneurons. Decerebrate, acutely spinalized cats have also been studied. All preparations were paralyzed with Flaxedil.

Under the influence of L-DOPA, both chronic and acutely transected cats demonstrated rhythmic neural bursting patterns of flexor and extensor filaments innervating the musculature of the ankle. The cycle time was short in the chronic animals (ranging from 0.75 to 1.9s) as compared with the acute animals (3.7 to 9.4s). However, the cycle time in the chronic cats were more variable than in acutely spinalized cats. A higher rate of discharge of alpha motoneurons was observed in the chronic animals. Evaluation of 16 extensor motoneurons revealed mean discharge rates of 41.3ms±22(SD), with a mean mode of 130±92.5ms. Successive interspike interval durations in a burst were highly variable. Initial doublet (< 10ms) discharges were seldom observed, however, these extremely short instantaneous intervals were commonly observed throughout the bursts in chronic but not acute cats. Irregular membrane potential fluctuations were routinely observed both during burst discharge periods and during interburst periods in the chronic animals. These consisted of sporadic hyperpolarizations and depolarizations. Neurograms of flexors and extensors were less coordinated in the chronic compared to the acute preparation. Simultaneous bursting, as well as some apparent randomly occurring periods of activity were observed in extensors and flexors in the chronic but not acute cats.

L-DOPA induced fictive locomotion can be demonstrated in the chronic, two week spinalized adult cat. Although the spinal neural networks responsible for fictive locomotion are present in these animals, there is evidence that significant modification of these networks has occurred. This research was supported by NINCDS grant NS16333.

275.5 EFFECT OF STRYCHNINE ON CORTICALLY INDUCED RHYTHMIC JAW MOVEMENTS IN THE GUINEA PIG. S.H. Chandler, L.J. Goldberg, S. Nielsen* and A. Hassel*. Depts. of Kinesiology, Oral Biology, Anatomy and Brain Research Institute, UCLA, L.A., CA, 90024

We have been interested in elucidating the neuronal mechanisms responsible for the production of rhythmical jaw movements (RJMs) which occur in the anesthetized guinea pig. Recently, Chandler and Goldberg (J. Neurophysiol., 48:126, 1982) have proposed that RJMs induced by repetitive cortical stimulation (RCS) in the ketamine anesthetized guinea pig are produced by the activation of a central pattern generator (CPG) which modulates a short latency cortico-brainstem-motoneuronal pathway to jaw-opener and closer motoneurons. The present study was designed to elucidate the role of glycine mediated inhibition in either the cortico-brainstem-motoneuron pathway or the CPG.

In 4 albino guinea pigs (400-600g) anesthetized with ketamine HCL (100mg/kg), RJMs were induced by repetitive stimulation (40Hz) of the cortical masticatory area. Stimulating electrodes were also implanted in the tongue to induce the jaw opening reflex and simultaneous inhibition of jaw closer motoneurons. Stimulating electrodes were also implanted in the mesencephalic nucleus of V to induce the monosynaptic jaw closing reflex. Prior to strychnine (STR) administration (0.1mg-0.6mg/kg i.v.) short pulse train stimulation of the masticatory area of the cortex (3 pulses, 500Hz, 0.3ms), or single shocks to the tongue, induced suppression of the jaw closing reflex at short (<10msec) conditioning-test intervals. Within 2 minutes after administration of STR, the suppression induced by short pulse train stimulation of the cortex was blocked and a direct activation of jaw-closer motoneurons was observed. The inhibitory effect of the tongue stimulation on the jaw closing reflex was only slightly reduced. Furthermore, the tonic excitability of the jaw closing motoneurons was increased as evidenced by the tonic increase in amplitude of the jaw closing reflex. During this period RCS still initiated RJMs which were larger in amplitude and higher in frequency than RJMs induced prior to STR administration. In most cases, during cortically evoked RJMs the stimulus evoked a co-activation of the jaw closer and opener muscles; this was never observed during RJMs prior to STR administration. These data further support our proposal that: 1) the inhibitory interneurons to jaw-closer motoneurons are not components of the CPG and, 2) cortically induced inhibition masks a short latency excitatory polysynaptic pathway from cortex to jaw-closer motoneurons. Furthermore, the data suggests that the masticatory CPG might not be critically dependent on glycine mediated inhibitory neurons for the production of RJMs.

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275.6 THE UPTAKE AND ELIMINATION OF METHYLPREDNISOLONE FROM THE 400 GM-CM CONTUSED CAT SPINAL CORD FOLLOWING A SINGLE 30 MG/KG I.V. INJECTION OF THE SODIUM SUCCINATE ESTER. J.M. Braughler and E.D. Hall. Program in Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

Studies from our laboratories have demonstrated profound biochemical and electrophysiological effects of large (15-90 mg/kg) i.v. doses of methylprednisolone sodium succinate (MP) on the normal cat spinal cord that we have hypothesized would be of benefit to the injured cord (Braughler and Hall, J. Neurosurg., 56: 838, 1982). In that report, we also described the pharmacokinetics of MP in the normal spinal cord and correlated tissue levels of MP with pharmacological actions. In a more recent study, we have shown that single 15 or 30 mg/kg i.v. doses of MP can significantly reduce lipid peroxidation and enhance $(Na^+ + K^+)$-ATPase activity in the 400 gm-cm contused cat spinal cord (Hall and Braughler, J. Neurosurg., 57, 1982, in press). In this report we describe the tissue pharmacokinetics of MP in the injured cord.

Cats of either sex (1.8-4 kg) were anesthetized with α chloralose (80 mg/kg i.v.), paralyzed with gallamine triethiodide (3 mg/kg i.v.) and the lumbar spinal cord from L2-L5 was exposed via dorsal laminectomy. The lumbar cord at L4 was subjected to a 400 gm-cm injury. Blood pressure and dorsal column evoked potentials were monitored throughout the course of the experiment. Cats received a single i.v. 30 mg/kg bolus of MP at various times after injury up to 8 hr, and injured (L4) and control (L2) segments were rapidly removed and frozen in liquid N_2 at various times after MP administration. Tissue MP was extracted and quantitated using normal phase HPLC as described earlier (McGinley, Braughler and Hall, J. Chrom., 230:29, 1982).

If MP was administered during the first 30 min after injury, roughly twice as much MP accumulated in the injured cord segment within 5 min compared with control. Less MP was taken up by the injured segment if MP was administered after 1 hr post-injury compared with administration at 5 or 30 min post-injury. MP given at 30 min post-injury accumulated slowly in the injured segment, but reached a peak concentration roughly 4 times that found in the control segment at 1 hr post-administration. Following this peak at 1 hr, MP was rapidly eliminated from the injured segment during the next hour with a $t_{\frac{1}{2}}$ of 45 min. This rapid elimination phase was then followed by a slower elimination phase having a $t_{\frac{1}{2}}$ of approximately 7 hr. MP was eliminated from the control segment with a constant $t_{\frac{1}{2}}$ of 5 hr. The study suggests that the tissue pharmacokinetics of MP in the injured segment differ significantly from those in uninjured tissue. It is anticipated that such pharmacokinetic data will provide a rational basis for the design of a more appropriate MP regimen for the effective treatment of human spinal cord trauma.

275.7 RESPONSES OF HYPOGLOSSAL MOTONEURONS TO VARIOUS NEUROTRANSMITTERS IN A BRAIN SLICE PREPARATION. R. A. Gregg and D. O. Carpenter, Ctr. Labs. Res., NY State Dept. Health, Albany, N. Y. 12201.

Motoneurons of the 12th cranial nerve nucleus were tested for their electrical responses to a variety of ionophoretically applied neurotransmitters. All experiments were performed on in vitro brain slices of the rat brain stem. Coronal sections (300 μm) were prepared by cutting through the excised brain stem at the level of the area postrema (an easily distinguishable landmark) and incubated in Kreb's-Ringer solution at 37°C for 1 hour prior to recording. Following the incubation period, single slices were transferred to a perfusable, total immersion chamber, secured by a fine nylon net and anchored in place with silver wire weights.

The hypoglossal nucleus, when viewed under 20x magnification, contrasted strikingly against the surrounding brain stem tissues. A 7 barrel ionophoretic electrode was positioned over the nucleus and driven into the tissue. The drug barrels were filled with varying combinations of 1M histamine (Hist), GABA, norepinephrine (NE), acetylcholine, (ACh), phenylephrine (PE), glutamate (Glu) and kainic acid (KA). The center barrel, filled with 2.5M NaCl, was used for recording.

The location of single units was facilitated by pulsing with Glu. In selected experiments the backing polarity current of Glu was reversed, evoking maintained spontaneous activity. At the termination of the recording pontamine sky blue was deposited at selected recording sites and the sites subsequently verified histologically by H and E staining in reference to the atlas of Pellegrino, et al. (1).

Both Glu and KA were potent excitatory agents at 50 nC on all units tested. The KA excitation typically was of a longer duration than Glu. Hist (100 nC) produced delayed but prolonged excitation of all units tested. This action of Hist was not depressed by 10^{-3}M metiamide. NE had excitatory action on most units similar to Hist. PE mimicked the NE actions, suggesting an alpha-1 receptor. In presence of low levels of Glu, tonic discharge was markedly depressed by GABA and ACh at 50 nC or less. Since it has been reported that there is considerable QNB binding to the hypoglossal nucleus (2) it is likely that the binding is a reflection of this inhibitory receptor.

These results demonstrate that it is possible to study cranial motoneurons in a brain slice, something which to date has not to our knowledge been possible for spinal motoneurons. Thus, this preparation holds considerable promise for elucidation of the ionic and metabolic mechanisms of transmitter action in mammalian motoneurons.

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275.8 CAN MOTOR UNIT TYPE BE PREDICTED ON THE BASIS OF THE MOTONEURON'S MEMBRANE PROPERTIES? J.E. Zengel, J.B. Munson, G.W. Sypert and S.A. Reid*. Univ. of Fla. Coll. of Med., Gainesville, FL 32610.

Motoneuron membrane properties can differ significantly among the different motor unit types. We wished to examine the possibility of using motoneuron membrane properties to determine motor unit type, which has traditionally been defined on the basis of muscle unit mechanical properties.

Our control data set consisted of 35 MG motor units which were classified on the basis of their mechanical properties into FF, FR and S motor units (1) and for which we had values for motoneuron conduction velocity, rheobase, input resistance (R_N) and after-hyperpolarization half-decay time. We examined the ability of various combinations of these motoneuron properties to predict motor unit type using a discriminant analysis computer program (2). This program takes a set of observations containing independent variables (the motoneuron membrane properties) and a classification variable whose values define groups for the observations (motor unit types), and uses these to develop a discriminant model that then classifies each observation into one of the groups.

The model was able to properly classify all 35 motor units of the control data set using the 4 membrane properties listed above. Rheobase and R_N together were also able to predict motor unit type for all 35 cells; other combinations of 2 or 3 variables yielded over a 90% accuracy in predictions. The best individual predictor of motor unit type was rheobase, which correctly identified motor unit type in 33 of the 35 cells.

Once the classification criteria are developed for one set of data, the discriminant model can then be applied to other data sets, thus allowing a further test of the model's ability to predict motor unit type. When the model developed for the control data set was tested against another independently obtained data set, 38 of the 41 cells in the second data set were properly classified. The 3 cells which were improperly identified were S units which the model classified as FR units. Upon examining the properties of these 3 units, we found that all had contraction times of just over 35 msec (the criterion used to distinguish slow from fast twitch units), suggesting that these cells may belong to a small subset of cells with properties intermediate between those of the FR and S motor unit types. As with the control data set, rheobase was the best individual predictor of motor unit type, properly classifying 35 of the 41 cells of this data set.

We conclude that, with the exception of a small subset of cells with intermediate membrane properties, it may be possible to classify motor units on the basis of the membrane properties of the motoneuron.

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275.9 LACK OF FIRM RELATION BETWEEN MOTONEURON SIZE AND MUSCLE UNIT MECHANICAL CHARACTERISTICS IN CAT EXTRAOCULAR MOTOR UNITS. S.J. Goldberg and J.R. McClung*. Dept. Anat., Med. Coll. VA.-VCU, Richmond, VA. 23298.

Antidromically identified motoneurons of the cat principal abducens nucleus were intracellularly stimulated in order to activate lateral rectus muscle single muscle units and were injected with horseradish peroxidase (HRP) for morphological identification and analysis. The structural characteristics of each neuron could then be correlated with the strain gauge measured force characteristics of the muscle fibers it innervated.

The HRP filled neurons ranged in size from 33.0 to 52.5 μm in diameter and 812 to 2229 μm^2 in area, as determined using a Zeiss image analyzer. The ranges of muscle unit force characteristics were as follows: Twitch tension - 4.99-293.3 mg, Maximum tetanic tension - 59.8-1278.48 mg, Twitch contraction time - 3.53-8.24 msec, Fusion frequency - 100-187 Hz. In addition to the above listed parameters for the twitch contracting motor units, we encountered one non-twitch unit which fused below 50 Hz and had a maximum tetanic tension of 49.0 mg. Of the twitch contracting muscle units, the most powerful was innervated by the largest motoneuron and the weakest was innervated by the smallest motoneuron. Besides this motoneuron size to muscle unit force correlation at the extremes of the sample we found no clear trends relating cell size to mechanical properties. An example of this lack of correlation is that motoneurons of 44.6, 42.1 and 38.9 μm in diameter innervated muscle units with maximum tetanic tensions of 119.65, 49.0 (non-twitch contracting) and 642.17 mg respectively.

It is clear that our present sample should be expanded to further investigate any possible trends in the data, but it seems apparent that cell body size does not correlate strictly with muscle unit mechanical properties. We are presently extending our investigation to include measurements of the entire cell surface area including dendritic tree, plus an examination of muscle unit fatigue properties and rheobase measurements of the motoneurons. We anticipate that this information will add to our understanding of structure-function relationships in this final common pathway system.

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275.10 COMPARTMENTALIZATION OF MUSCLE UNITS IN CAT LATERAL GASTROCNEMIUS MUSCLE. A. W. English and O. I. Weeks*. Dept. of Anatomy, Emory University, Atlanta, GA 30322.

Cat lateral gastrocnemius (LG) muscle is organized into four compartments, each of which is supplied by a primary branch of the LG muscle nerve. To determine the relationship between this compartmental organization and the organization of LG motor units, the distribution of muscle fibers associated with individual, type-identified motor units (muscle units) was examined. Single motor units were isolated from dissected ventral root filaments and the mechanical properties of their muscle units used to classify them according to the nomenclature of Burke et al. (J. Physiol. 238: 503-514, 1973). Preliminary localization of the muscle unit was made from electromyograms (EMG's) recorded from bipolar electrodes implanted in each compartment. Individual units were then stimulated according to a protocol known to deplete their muscle fibers of glycogen (Burke et al., op cit), and the distribution of these muscle fibers was determined from serial reconstruction of cryostat sections stained for the periodic acid-Schiff (PAS) reaction. In all cases the fibers from single muscle units were confined within the boundaries of compartments. Compartmental localization based on EMG recordings was consistent with these anatomical findings. Muscle fibers of the large type FF motor units were usually found throughout the entire subvolume of a compartment. Smaller type FR and S motor units supplied muscle fibers restricted to a compartment, but these were not always distributed throughout the entire compartment. Thus each LG compartment contains a distinct population of single motor units.

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275.11 CENTRAL ORGANIZATION OF CAT LATERAL GASTROCNEMIUS MOTOR UNITS. O. I. Weeks* and A. W. English (SPON: B. Brown). Dept. of Anatomy, Emory University, Atlanta, GA 30322

The distribution of motor neurons which supply primary branches of the cat lateral gastrocnemius (LG) muscle nerve was examined in order to determine whether the motor neurons which supply different LG compartments are topographically organized. Individual muscle nerve branches to LG were isolated by microdissection and soaked in 30-50% solutions of horseradish peroxidase (HRP) in 1.6 mg % hyaluronidase. As a control, in each case, the entire contralateral LG nerve was cut and soaked. The branch to m. soleus was left intact. Animals were sacrificed after 72 hour survival periods. The L_6-S_1 segments of the spinal cord were sectioned serially and then reacted for demonstration of HRP using tetramethylbenzidine (TMB) as a chromagen. Retrogradely labeled motor neurons were identified on both sides of the cord, and the spatial distribution of neurons supplying the different nerve branches was compared to the distribution of the entire contralateral LG motor nucleus. Neurons supplying proximal compartments of the muscle tend to occupy more rostral portions of the LG motor nucleus, and neurons supplying the distal most compartment are distributed to the caudal part of the pool. Thus, although significant overlap was found in the distribution of labeled neurons supplying different branches, a clear topographic organization is indicated.

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275.12 **AFFERENT AND EFFERENT COMPONENTS OF THE VAGUS NERVE IN FROG.** S.L. Stuesse, W.L.R. Cruce, and K.S. Powell*. Neurobiology Program, N.E. Ohio College of Medicine, Rootstown, OH 44272.

Previous investigations from our laboratory have indicated that in rat, the majority of cells projecting to the myocardium orginate in the nucleus ambiguus of the medulla. In the frog, the vagus nerve branches to supply major organs of the body, including the heart, but frogs have been reported to have no nucleus ambiguus. The brainstem origin of the frog vagus nerve has been referred to as the IX-X complex. We investigated the afferent and efferent components of major vagal branches by using horseradish peroxidase (HRP) as a tracer. In 24 frogs, *Rana Pipiens*, identified vagal branches were cut and dipped in a 30-40% HRP solution for 1-2 hours. The cardiac, gastric, laryngeal and pulmonary branches of the vagus were each soaked. After 1-2 weeks, the frogs were sacrificed and processed for HRP-reaction product using the tetramethylbenzidine method. Vagal afferents entered with the vagus nerve and descended in two tracts. The majority entered the lateral aspect of the tractus solitarius and descended to cervical spinal cord while slowly crossing to a ventromedial position within the tract. A smaller portion of afferents descended in the dorsolateral funiculus with the spinal tract of V to thoracic spinal cord levels. The laryngeal branch contained few afferents. A column of vagal efferent cells was identified in a ventrolateral position from the level of the brainstem exit of vagus nerve (3000um above the obex) to approximately 200um below the obex (values given are for a frog weighing 80gm). This cell group was separate from and just caudal to efferent cells of the glossopharyngeal nerve. There was some topography apparent within the nucleus. Cells projecting through the gastric branch were found throughout the rostral-caudal extent of the nucleus. However, cardiac cells were clustered rostral to "pulmonary" cells, and "laryngeal" cells were located caudally in the nucleus. No evidence for a separate ventrolateral cell group which would correspond to a nucleus ambiguus was found.

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276.1 FUNCTIONAL ORGANIZATION OF THE PRIMATE PUTAMEN. M. D. Crutcher and M. R. DeLong. Depts. of Physiology, Neurology and Neuroscience, Johns Hopkins Sch. of Med., Baltimore, Md. 21205.

In the primate, the putamen receives dense, topographically organized projections from the motor and sensory cortices, suggesting that the putamen may be somatotopically organized. However, no attempt has been made to characterize systematically the somatotopic organization of the putamen, or to study the relation of neuronal activity in the putamen to active movements and natural somatosensory stimulation in the awake, intact primate.

Two rhesus monkeys were trained to permit passive manipulation. Sensory stimulation consisted of passive joint rotation, tendon and muscle taps, and light touch to the hairy and glabrous skin. In addition, the relation of neuronal activity to spontaneous movements and active movements elicited by the presentation of food objects was observed.

Neurons (N=707) were categorized on the basis of their relation to active movements or responses to sensory stimulation of individual body parts. 38% of neurons were related to the arm, 9% to the leg, 11% to the mouth or face, and 3% to axial portions of the body. The remaining neurons exhibited non-specific activation which could not be confidently localized to an individual body part (13%), or did not change their activity during the examination (26%). A large proportion (41%; N=270) of the "arm" neurons were responsive to somatosensory stimulation. For these neurons the most effective sensory stimulus (82%) was passive joint rotation. Only 6 (5%) of these arm neurons responded to cutaneous stimulation.

The putamen was found to be somatotopically organized. Each body part was represented over a long anteroposterior extent of the nucleus. Clusters of 2-5 neurons with similar relations to active movements or responsive to passive movements of a single joint were often encountered over a 100-500 micron distance. Clusters of neurons with sensory driving were organized by joints. Rather than a single elbow or shoulder area, multiple clusters of neurons related to each joint were widely distributed over a long anteroposterior extent of the nucleus and were adjacent to clusters of neurons related to other joints of the arm. These clusters of neurons with similar functional properties may correspond to the patches of terminal labelling of the cortico- and thalamostriate projections and the patches of acetylcholinesterase activity and enkephalin immunoreactivity which have been described for the striatum. We propose that these clusters of neurons with similar functional properties represent the basic functional units of the striatum in a manner analogous to the funcional columns of the neocortex.

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276.2 **LEARNING AND MOVEMENT AFFECT SENSORY PROCESSING IN THE BASAL GANGLIA.** **T.I. Lidsky and J.S. Schneider S.U.N.Y., Stony Brook, N.Y. 11794**

Previous work from this laboratory described some of the information encoded within tactile sensory inputs into the striatum and globus pallidus of cats. Stimulus location and direction of movement were represented. However, this was not done absolutely as in the primary somatosensory system. Rather location and direction of movement of a stimulus was referenced to the front of the mouth ("orocentric").

Orocentric information would be useful in guiding head movements that bring the evoking stimulus to the mouth (such as in biting during predation). However, that only orocentric responses were observed is perplexing-not all head movements are intended to align the evoking stimulus with the mouth. If orocentricity were "hard wired" into the basal ganglia (BG), the behavioral adaptability of this neural system would be severely limited. For this reason, the plasticity of BG sensory processing was investigated.

Learning had an important influence on BG responsivity. Cats were taught to recline quietly during recording sessions by rewarding appropriate behavior with milk delivered through a tube resting against the animal's upper lip. With increasing experience in the recording chamber, animals remained quiet for progressively longer periods. Perhaps more important, the behavioral significance of the perioral stimulus engendered by the milk tube became more obvious. In parallel with the cat's increasing experience, progressively higher proportions of striatal cells were observed to have perioral receptive fields.

Movement also influenced BG sensory responses. Presentation of perioral tactile stimuli during jaw movement evoked enhanced responses as compared to equivalent stimuli presented in the absence of movement. This enhancement of perioral sensitivity was not due to arousal. Pupillary dilatation and nuchal EMG indicated that the animal was aroused during movement and non-movement conditions.

A small proportion of BG cells (about 5% of responsive sample) only had sensory responsiveness under certain conditions. These units did not fire in response to orofacial stimuli presented when the animal was at rest. In addition, these cells did not fire in relation to jaw movements. However, if orofacial stimulation was presented during jaw movement, pronounced firing rate changes occurred.

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276.3 THE BASAL GANGLIA AND HEAD MOVEMENT: INFLUENCES ON SENSORY INPUT TO CERVICAL MOTONEURONS. U.E. Olazábal and T.I. Lidsky, S.U.N.Y. Stony Brook, N.Y. 11794. Striatopallidal lesions in rats disrupt head and trunk movements. When required to lick a recessed drinking tube, animals typically position the head inaccurately. On these few occasions in which the head is initially aligned with the tube, postural fixation is not achieved and the head drifts out of the proper orientation. In addition, similar difficulties in alignment of the body also occur after striatopallidal lesions. These problems are not due to a simple motor deficit since seemingly normal head and trunk activities occur during grooming, locomotion and rearing. Deficits are only obvious when the motor responses must be guided by somatosensory feedback (from perioral contact with the drinking tube).

Several investigators have shown direct, perhaps monosynaptic projections from trigeminal primary sensory afferents to cervical motoneurons (cf.1). It has been suggested that this pathway underlies orienting movements of the head which are guided by somatosensory feedback (1). In view of the somatosensory component in striatopallidal lesion-induced postural difficulties, the influence of the basal ganglia (BG) upon trigeminally-evoked cervical motor responses was assessed.

Stimulation electrodes were chronically implanted in maxillary perioral skin. EMG electrodes were sutured into the splenius muscle-the primary agonist for lateral head flexion. Single pulse stimulation reliably evoked a short latency (4 msec) splenius response. Thresholds were calculated repeatedly over a two to three day period and were seen to be quite stable. Striatopallidal lesions were then produced bilaterally and thresholds were again assessed. BG lesions produced a large (40-50%) drop in threshold. Moreover, stimulation intensities, which affected only neck muscles in the intact rat, evoked responses in thoracic and lumbar axial muscles after BG damage. Thresholds remained low for at least ten days.

In summary, BG lesions disrupt head and trunk movements that are made under somatosensory control without affecting movements made in different contexts. Similar brain damage alters the excitability of the pathway by which trigeminal sensory afferents directly affect cervical and trunk axial motoneurons. Previous work from this laboratory showed that changes in sensory-evoked trigeminal motor responses after BG damage were due to influences on sensory rather than motor elements (2). Considered altogether, these data suggest that the BG's role with respect to movement in general involves influences upon sensory processing. (Supported by NINCDS Grant #16054).

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276.4 [^{3}H]-SPIROPERIDOL RECEPTOR BINDING IS RELATED TO SPEED OF MOVEMENT INITIATION IN YOUNG AND OLDER ADULT RATS. W. W. Spirduso, P.E. Gilliam, and R.E. Wilcox, Dept. of Physical and Health Ed. and School of Pharmacy. The Univ. of Texas, Austin, TX 78712.

A relationship between rapid movement initiation and the nigrostriatal dopamine (DA) system has been suggested by results from studies of Parkinson's disease, aging, and pharmacological manipulations of the nigrostriatal DA system. For example, chlorpromazine, a known DA antagonist, was found to slow movement initiation in a reactive capacity task (Spirduso et al., 1981). Wolf, Wilcox et al. (1980) reported that a fast reacting CR-CD/F strain was also higher in [^{3}H]-spiroperidol receptor binding, while a significantly slower Zivic-Miller strain was correspondingly lower in [^{3}H]-spiroperidol receptor binding. In this experiment, the relationship between the nigrostriatal DA system and speed of movement initiation is more rigorously tested by comparing the fastest responders to the slowest responders in a within-strain design. Further, the question is asked as to whether this relationship would be more easily revealed in older adult rats that have decreased DA binding (Govoni et al., 1978).

Thirty young (Y=90 days) and 30 old (O=540 days) male Fisher 344 rats were operantly conditioned in a reaction time type task (50 trials a day for 7 days). This task requires extremely fast ballistic responses on four tasks, each requiring progressively faster responses to meet the criterion for success (1000, 300, 200 msec). The caudate nuclei from the 6 fastest (F) and the 6 slowest (S) responders from each age group were assayed for [^{3}H]-spiroperidol binding (Riffee, Wilcox, et al. 1982).

The fastest rats in both age groups were significantly higher in specific [^{3}H]-spiroperidol (0.2nM) binding than the slowest rats ($\bar{x}_F$=12,749,$\bar{x}_S$=7,178 dpm/mg protein; $F(1,20)$ 27.18 = $p < .01$).[^{3}H] receptor binding density was also significantly lower in the old rats ($\bar{x}_Y$=15,561 vs $\bar{x}_O$=4,365 dpm/mg protein; $F(1,20)$ 109.76 = $p < .01$). No age effects were seen in either percent success or speed of responses, indicating that when the fast rats are selected from a sample of old rats, they respond as quickly as the fastest young rats. It appears that in the old rats where receptor binding was maintained no behavioral deficits were seen. It is suggested that, as in the Wolf et al. (1980) results, increased receptor density (Bmax) may serve as a compensatory mechanism to maintain behavioral performance.

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276.5 EXTRINSIC AND INTRINSIC ORIGIN OF GAD IN PARS COMPACTA OF THE RAT SUBSTANTIA NIGRA. I. Grofova* and F. Fonnum*. (SPON: W. Falls). Dept. of Anatomy, Michigan State University, East Lansing, MI 48824 and Norwegian Defense Research Establishment, Kjeller, Norway.

Recent reports have provided evidence that axons of the substantia nigra pars reticulata projection neurons emit several intrinsic collaterals which arborize in both pars compacta (SNC) and pars reticulata (SNR) of the substantia nigra (Grofova et al., J. Comp. Neurol., in press; Karabelas and Purpura, Brain Res. 200:467, 1980). The transmitter substance in these collaterals is likely to be γ-aminobutyric acid (GABA) since the nigrothalamic and nigrotectal pathways arising in the SNR were shown to be GABAergic (Di Chiara et al., Brain Res. 176:273, 1979). To test this hypothesis, we examined the concentrations of glutamic acid decarboxylase (GAD), the enzyme synthesizing GABA, in the SNC and SNR of rats submitted to kainic acid (KA) lesions of the SNR.

Concentrations of GAD were measured by biochemical assay in samples dissected under a stereomicroscope from freeze-dried sections. A precise dissection of the SNC appeared to be of critical importance since contamination of the samples by tissue located dorsally or ventrally to the cellular layer of SNC considerably influenced the biochemical data. Therefore, cases with large SN lesions obscuring the distinction between SNC and SNR were discarded. The dissection was histologically controlled for all samples. The loss of SNR cells was associated with 30-40% decrease of GAD activities in the SNC as compared to the normal side. However, there were only insignificant changes in GAD concentration within the lesioned SNR. In contrast, hemitransections of the brain rostral to the SN which interrupted all or the majority of descending nigral afferents resulted in a large reduction in GAD activities within the SNR (up to 90%), while the SNC showed an average of only 65% reduction.

These results suggest that a significant proportion of GAD in the SNC is derived from intrinsic sources, such as axon collaterals of the SNR projection neurons.

276.6 CYCLIC NUCLEOTIDE DISTRIBUTION IN RAT STRIONIGRAL NEURONS: RELATIONSHIP TO GAD- AND SP-CONTAINING ELEMENTS. S.K. Ufkes* & M.A. Ariano (SPON: M. Moffroid). Anatomy & Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Identification of specific strionigral efferent neurons has been accomplished using retrograde transport of the fluorescent dye, Evans blue (EB). 48-72 hours following stereotaxic injection of 0.2 µl of a 40% solution of the dye into the substantia nigra, rats were sacrificed by transcardiac perfusion with cold 4% paraformaldehyde, 0.5% glutaraldehyde, 0.25 M sucrose in phosphate-buffered saline, pH 7.2. 8 µm frozen sections were processed for cyclic nucleotide immunohistochemistry (Ariano, et al, Neuroscience 5: 1269, 1980) or the neurotransmitter immunoreactivity of substance P (SP) or GABA.

Tissue sections were examined with ultraviolet optics using a 546 nm primary filter/590 nm secondary filter to ascertain EB-labeled strionigral neurons. EB-labeled neurons were brilliant red and had oval or rounded perikarya. The excitation/barrier filter system was changed to enable visualization of the fluorescein fluorophor employed to discern antibody immunoreactivity of the cyclic nucleotides, cyclic AMP and cyclic GMP, or SP or GABA containing elements within the rostral striatum (primary filter 485 nm/secondary filter 520 nm). 28.9% of cyclic AMP-immunoreactive elements were identified as strionigral efferent neurons, and 46% of the strionigral neurons contained cyclic AMP. 38.5% of the cyclic GMP-immunoreactive cells were identified as strionigral neurons, and 81.5% of the strionigral neuron population contained this cyclic nucleotide.

The identified neurotransmitters employed by the strionigral pathway are SP (Mroz, et al, Brain Res. 125: 305, 1977) and GABA (Kim, et al, Brain Res. 14: 45, 1971). SP immunoreactive cells in comparable regions of rat striatum were ovoid, lacked nuclear fluorescence, and averaged 15 µm in diameter in their largest dimension. SP-like immunoreactivity was assed by application of a monoclonal antibody (Cuello, et al, PNAS USA 76: 3532, 1979). GABA-containing cells were discerned by GAD (EC 4.1.1.15) immunohistochemical localization using a specific sheep polyclonal antibody (gift, I.J. Kopin). GAD immunoreactivity was localized within ovoid and rounded neurons, 15-20 µm diameter in the rostral caudate-putamen complex. The cytoarchitecture of the GAD-containing and SP-reactive neurons is coincident with the immunofluorescence of cyclic GMP stained neurons. Cyclic AMP stained neuronal elements are very different in appearance from SP-positive and GAD-reactive neuronal somata.

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276.7 NEOSTRIATAL ACETYLCHOLINESTERASE ASSOCIATED WITH DOPAMINERGIC AFFERENTS. J. F. Marshall, M. R. Kozlowski, and K. G. van Oordt*. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717

Although the dopamine (DA)-containing neurons in the pars compacta of the substantia nigra and ventral tegmental area show moderate staining for the enzyme acetylcholinesterase (AChE), the axon terminals of the nigrostriatal projection are not believed to contribute to the intense cholinesterase staining of the neostriatum of the rat. Damage to the DA afferents to the neostriatum has been shown not to decrease the visible cholinesterase staining of the neostriatum, as revealed by the histochemical methods of Koelle (J. Comp. Neurol., 100:211, 1954) or Karnovsky and Roots (J. Histochem. Cytochem., 12:219, 1964). We have used the Herkenham-Pert (Nature, 291: 415, 1981) modification of the Hardy et al. (Neurosci. Lett., 3:1, 1976) AChE procedure, using unfixed tissue and iso-OMPA to suppress nonspecific cholinesterase enzyme activity. Using this procedure, we find that the neostriatum and related limbic structures (nucleus accumbens septi, NAS; olfactory tubercle, OT; amygdaloid nuclei) stain intensely, whereas most other forebrain structures show no or faint reaction. Within the neostriatum, islands of lighter stain (striosomes) are evident against the darker background.

Rats were given unilateral ventral tegmental injections of 6-OHDA. After 1, 3, 7, or 28-42 days the rats were killed by decapitation. The brains were sectioned (20-30 µm) in a cryostat (-15 °C). Adjacent sections were stained for AChE using the procedure of Hardy et al. or Karnovsky-Roots. Optical density measurements were used to quantify the intensity of the staining.

In animals sacrificed at least 3 d postoperatively, the Hardy et al. method revealed significantly decreased staining in the neostriatum and NAS ipsilateral to the lesion, relative to the contralateral control hemisphere. This lesion-induced decrease was most obvious in the caudal one-half of the neostriatum. More rostrally, a decline in AChE staining was also evident in the medial (periventricular) neostriatum and in the dorsolateral NAS of most lesioned animals. These hemispheric asymmetries were not evident at 1 d postoperatively, when little neostriatal DAergic terminal degeneration has occurred. Using the Karnovsky-Roots procedure, striatal or NAS AChE asymmetries were not readily visible.

These results indicate that (1) part of the neostriatal AChE depends on the integrity of the dopaminergic innervation (and, likely, is localized to the dopaminergic axon terminals), (2) the AChE staining associated with the dopaminergic afferents shows a marked regional localization within the neostriatum similar to that of CCK immunohistochemistry, and suggest (3) that some of the dopaminergic axon terminals in the neostriatum could be cholinoceptive.

276.8 OVARIECTOMY ENHANCES INTRASTRIATAL DOPAMINE-INDUCED CONTRALATERAL DEVIATION. R. L. Smith, L. T. James*, J. N. Joyce and C. Van Hartesveldt. Psychology Department, University of Florida, Gainesville, Florida 32611

In previous research we have shown that in the male rat, systemic injections of estradiol benzoate initially suppress intrastriatal dopamine (DA)-induced contralateral postural deviation (Joyce, et al., Soc. Neurosci. Abstr. 7:781, 1981). To determine whether the influence of endogenous estrogen has a similar effect, we examined the effects of ovariectomy (OVX) on intrastriatal DA-induced contralateral postural deviation.

Female Long-Evans hooded rats were bilaterally implanted with 21 GA stainless steel guide cannulae, positioned in the dorsal anterior caudate-putamen (CPU). Unilateral injections of drugs were made with a 27 GA injection cannula. Each animal received an injection of the vehicle solution (.25 µl of .3M sodium phosphate dibasic) in the morning and the drug (DA, 25 µg in .25 µl buffered solution) 4 hours later. After each injection the animal was placed in a circular chamber and observed for 30 minutes. Duration of time spent in ipsilateral or contralateral postural deviation was recorded. Animals were given bilateral OVX after completion of the first trial and were tested again in the same manner 2 days and 7 days after OVX.

Consistent with our previous results using male rats, the female rats showed contralateral postural deviation in response to intrastriatal injections of DA but not to the vehicle solution (Joyce, et al., Eur. J. Pharmacol. 72:1-10, 1981) Two days after OVX, DA elicited greater contralateral deviation than in the pre-OVX test. Seven days after OVX the behavioral response to DA had returned to pre-OVX levels.

We suggest that the enhanced response to DA at 2 days post-OVX might be due to release from the suppression of DA activity by estrogen. This effect, however, is short-lived and is reminiscent of changes found after withdrawal from DA antagonists.

276.9 THE ROLE OF PRESYNAPTIC SENSITIVITY IN THE MAINTENANCE OF STRIATAL DOPAMINE HYPERSENSITIVITY. J.H. Gordon and V.L. Radice*. Dept. Pharmacology, Univ. Hlth. Scis./The Chicago Med. Sch., N. Chicago, Il 60064.

Several hormonal and/or drug manipulations have been shown to result in the development of a permanent striatal dopamine hypersensitivity (SDH). The present study reports on the role of pre- and postsynaptic mechanisms in the development and/or maintenance of SDH. To distinguish between the neurochemical alterations involved in the development from those involved in the maintenance of SDH two different types of animals were studied. 1. animals that had displayed SDH for 3 months (i.e. longterm ovariectomized; LTX, and the hypophysectomized male; HM). 2. Short-term ovariectomized rats treated with either estradiol benzoate (EB; 100ug/kg-day X 3) or haloperidol (HAL; 1.0 mg/kg-day X 16) and sacrificed before and after the development of the "withdrawal" SDH. All animals with SDH displayed an increased B-max for ^{3}H-spiroperidol (^{3}H-spiro) binding to striatal (STR) membranes, and a decreased V-max for STR tyrosine hydroxylase (TH). The increased ^{3}H-spiro binding appears to be a neurochemical reflection of the SDH, while the decreased V-max for TH is probably a compensatory response to the altered postsynaptic dopamine sensitivity. The Kd values for ^{3}H-spiro remained constant for all groups, except the group that was treated acutley with EB and sacrificed prior to the development of the SDH. In this group the Kd value was increased which probably reflects the mechanism of action for EB's ability to decrease dopamine potency. All treatments resulted in a decreased Km for the pterine cofactor for TH. This reflects a short-term modulation of TH in response to the treatments and not a longterm response. The only parameter measured that appears to be associated with the maintenance of SDH was an increase in presynaptic sensitivity. Presynaptic sensitivity was measured by the ability of apomorphine to inhibit TH activity in synaptosomes. Utilizing this measure of presynaptic sensitivity only those animals with longterm SDH and the acute treatment with EB resulted in presynaptic hypersensitivity. Moreover the LTX animals show a time related decrease in glutamic acid decarboxylase (GAD) activity in the substantia nigra, which may be an indication of an altered neuronal feedback. This altered neuronal feedback and the increased presynaptic sensitivity indicate that the dopamine neurons have shifted the primary control of synthesis/release of dopamine to presynaptic mechanisms. Such a shift could be the neurochemical mechanism responsible for the maintenance of SDH, because a decrease in the synthesis/release of dopamine in the SDH could only exacerbate the postsynaptic hypersensitivity. This work was supported in part by a grant from NIMH (MH33991).

276.10 SPECIFIC REQUIREMENT FOR ESTROGENIC PROPERTIES TO ANTAGONIZE THE DEVELOPMENT OF NEUROLEPTIC-INDUCED DOPAMINE HYPERSENSITIVITY V.L. Radice* and J.H. Gordon (Spon: S. Ehrenpreis). Dept. Pharmacology, Univ. Hlth. Scis./The Chicago Med. Sch., N. Chicago, Il 60064.

Estradiol benzoate (EB) has been shown to antagonize the development of neuroleptic-induced striatal dopamine hypersensitivity (NI-SDH), when administered during the withdrawal from chronic neuroleptics. The present study was undertaken to determine if other steroid hormones could also antagonize the development of NI-SDH, and to test the responses of various estrogenic compounds.

Dose response curves indicated that EB was an effective antagonist for the development of the NI-SDH in doses as low as 2.5 ug/kg-day. While doses above 10.0 ug/kg-day were less effective than the 10.0 ug/kg-day dose. The decreased efficacy for the high doses of EB probably reflects the biphasic response of the dopamine receptors to EB, as high doses of EB can cause a compensatory increase in the postsynaptic potency of dopamine in response to the initial suppression of postsynaptic potency. The antiestrogens (α-clomiphene, t-clomiphene and CI-628) also antagonized the development of NI-SDH. These results are probably related to the fact that these antiestrogens are partial agonists and the effectiveness of low doses of EB indicate that minimal estrogenic stimulation is required to antagonize the response to the neuroleptics. 17-alpha-estradiol, testosterone propionate (TP), and dihydrotestosterone did not antagonize the development of the NI-SDH. The dose response curves for TP, depoprovera, and dexamethasone shifted the dose response curve for apomorphine in both directions in a non-dose dependent fashion. The preliminary binding data do not support a central site of action for these steroid effects, at least for the dopamine system as both the B-max and Kd for ^{3}H-spiroperidol appear to be unchanged relative to the animals treated with neuroleptics only.

The catecholestrogens (CE's; 2-hydroxyestrone and 2-hydroxyestradiol) caused an exacerbation of the NI-SDH. This increase in NI-SDH may be related to the ability of CE's to inhibit tyrosine hydroxylase (TH), thus compromizing presynaptic mechanisms during the compensatory responses in the postsynaptic cells. Production of CE's following high doses of EB could also account for the biphasic effects of EB on striatal dopamine receptors, as their production following high doses of EB could compromize presynaptic function during the rebound or compensatory phase that follows the initial suppression of postsynaptic dopamine potency.

In conclusion the ability of EB to antagonize the development of NI-SDH appears to be related to its estrogenic properties and not to the steroid nucleus or other hormone effects.

This work was supported in part by a grant from the NIMH (MH33991).

276.11 CORRELATION OF CHANGES IN DOPAMINE (D-2) RECEPTOR BINDING WITH APOMORPHINE-INDUCED STEREOTYPE BEHAVIOR AFTER ESTROGEN-INDUCED HYPO- AND HYPER-SENSITIVITY. J.Z. Fields, B.A. Anderson* and J.H. Gordon. Department of Pharmacology, Chicago Medical School, North Chicago IL 60064.

Estrogen (estradiol benzoate (EB), 100 ug/kg X 3 days, s.c.) administration to ovariectomized rats led to a decrease in apomorphine (0.4 mg/kg, i.p.) induced stereotype behavior at 24 h after the last dose of EB. At later time points after EB withdrawal (48, 72, 96 h) the animals had converted to a behaviorally hyper-sensitive state. Stereotypy scores for control, 24 h and 72 h groups were 3.1 +/- 0.2, 1.1 +/- 0.1 and 4.2 +/- 0.4, respectively. The stereotypy rating scale is non-parametric and the scores can not be directly used to yield a percentage change in behavior among groups.

Dopamine receptors (D-2) were labelled with (3H)spiroperidol and several binding parameters were determined. The receptor density of the high affinity, butaclamol sensitive site (Kd 20 to 50 pM) did not significantly change in the hypo-sensitive 24 h group but it did increase (+56%), consistent with the behavioral change, in the 72 h group. The lower affinity (3H)spiroperidol binding site (Kd greater than 250 pM) was demonstrable but was not further assayed for both technical and theoretical reasons. The affinity for antagonist, spiroperidol, did not change in the 72 h group but did decrease (-63%), consistent with the behavioral change, in the 24 h group.

Since changes in antagonist binding parameters are not always identical or even similar to changes in agonist binding parameters curves for inhibition of (3H)spiroperidol(100 pM) binding by a dopamine agonist, ADTN, were developed. The ADTN inhibition curves (of high affinity (3H)spiroperidol binding) can be further dissected into two sites of higher and lower affinity and these two sites can change in absolute or relative proportion and/or in absolute or relative affinity. Data accumulated so far suggest that changes in agonist binding are consistent with neither antagonist binding changes nor with changes in behavior. Implications for models of the D-2 receptor and its relation to stereotypy will be discussed. (These studies were supported in part by NIMH grant MH-33991)

276.12 INTRASTRIATAL ESTRADIOL SUPPRESSES APOMORPHINE-INDUCED POSTURAL DEVIATION BUT ENHANCES THAT OF AMPHETAMINE. J. N. Joyce, M. Ireland*, and C. Van Hartesveldt. Psychology Department, University of Florida, Gainesville, Florida 32611

Recent research on the interaction between estrogens and the nigrostriatal dopamine (DA) system have yielded discrepant results. Our own data (Joyce, et al., Soc. Neurosci. Abstr. 7:781, 1981) utilizing DA cannulation into the striatum suggests that estrogens suppress DA-related behaviors, while the results of Becker, et al. (Soc. Neurosci. Abstr. 7:42, 1981) and Robinson, et al. (Soc. Neurosci. Abstr. 7:42, 1981) indicate that estrogens enhance DA related behaviors. The present experiment was performed to determine whether these discrepancies might be resolved.

Female Long-Evans rats were ovariectomized and implanted bilaterally with permanent guide cannulae in the dorsal anterior striatum. After a 1 week recovery period, a 27 GA cannula which was either empty or contained 17β-estradiol, 17α-estradiol, or cholesterol was inserted into the guide cannula. Four hours later rats were injected I.P. with either 1 ml/kg isotonic saline, .75 mg/kg apomorphine, or 3.0 mg/kg amphetamine. Immediately after the saline or apomorphine and 20 min after the amphetamine, rats were observed for 30 min in a circular chamber. Duration of time spent in ipsilateral or contralateral postural deviation was recorded.

Rats implanted with 17β-estradiol showed no asymmetry in response to I.P. saline, but exhibited ipsilateral postural deviation to I.P. apomorphine. Cholesterol and 17α-estradiol were ineffective. Thus, 17β-estradiol suppresses the striatal postsynaptic response to a direct DA agonist. However, when rats were implanted with 17β-estradiol and given amphetamine I.P. they showed contralateral postural deviation. Thus 17β-estradiol enhances the effect of amphetamine, a drug which acts on the nigrostriatal DA terminals to release DA.

Our results suggest a way to integrate the discrepant findings with respect to the interaction between estrogens and dopamine. We suggest that estradiol suppresses the postsynaptic response to DA in the striatum. When this occurs, there is a reduction in the activity of the striatonigral GABA feedback loop (McGinnis, et al., J. Neurochem. 34:785, 1980) and a compensatory increase in the activity of the nigrostriatal DA neurons. Our results have demonstrated that estradiol both suppresses the postsynaptic response to DA agonists and enhances release of DA from nigrostriatal terminals simultaneously.

276.13 INTERACTIONS BETWEEN DOPAMINE AND SUBSTANCE P SYSTEMS IN RAT BASAL GANGLIA, G. Hanson, E. Gisclon*, M. Peat* and J. Gibb. Lab. of Biochem. Pharmacol. and Tox., College of Pharmacy, University of Utah, Salt Lake City, Utah, 84112.

The relationships between neuronal structures in the striatum and substantia nigra of the basal ganglia are known to be important in the regulation of posture and locomotion. An interaction between the dopaminergic (DA) nigral-striatal and the feedback substance P (SP) striatal-nigral systems of this neuronal circuitry has been described by Hong et al. (Neuropharm. 17 [1978] 83-85). To elucidate further this relationship we have examined the response of the SP striatal-nigral pathway to pharmacologically-mediated changes in striatal DA activity. The response of the SP loop to dopaminergic drugs was monitored by determining the levels of substance P-like immunoreactivity (SPLI) in the substantia nigras (site of the SP terminals) of treated and control rats. The radioimmunoassay technique used to measure the SPLI content was sensitive enough to detect 10 picograms of SPLI per sample and is described in detail elsewhere (Hanson, G. and Lovenberg, W., J. Neurochem., 35 [1980] 1370-1374). The levels of nigral SPLI were determined following DA antagonism by either subacute receptor blockade with haloperidol (1 mg/kg, daily; 2 wks.) or destruction of the nigral-striatal DA pathway with a nigral injection of 6-hydroxydopamine. Blockade of DA receptors resulted in a reversible 28% decrease in nigral SPLI while destruction of the DA neurons lowered nigral SPLI by 39%, 24% and 13% after 14, 21, and 28 days, respectively. In contrast, enhancement of the DA system with subacute amphetamine treatment (5 mg/kg, 2X daily; 2 wks.) increased the SPLI content of the substantia nigra by 23%. The SPLI levels were still elevated (24%) 2 weeks following the final amphetamine injection. Paradoxically, a single injection of amphetamine (15 mg/kg) significantly lowered nigral SPLI content (34%) 6 hours following drug treatment. With acute amphetamine administration, however, levels of SPLI in the substantia nigra had returned to normal by 24 hours post treatment.

276.14 LOCAL INFUSIONS OF SEROTONIN INTO THE NEOSTRIATUM: EVIDENCE FOR DISINHIBITION OF SURROUND. Stephen D. Curtis and George V. Rebec. Dept. of Psychol., Indiana Univ., Bloomington, IN 47405.

In the neostriatum, a topographic representation of the cerebral cortex overlaps with similarly patterned inputs from thalamus and substantia nigra (e.g., Grofova, The Neostriatum [Divac and Oberg, eds.], 1979). Feedback to these areas is also topographic. This system of reciprocal interconnections may allow the neostriatum to function as a "selective rheostat" which sets up areas of preferential information flow in specific regions (Iversen, The Neostriatum [Divac and Oberg, eds.], 1979). Describing a similar view of neostriatal function, Hassler (J. Neurol. Sci., 1978, 36:187) suggested that a specific, localized area of the neostriatum, which is activated by cortical input, is enhanced relative to other areas of the neostriatum by inhibitory interneuronal mechanisms. According to this view, a change in firing rate in one region of the neostriatum is accompanied by a reciprocal change in surrounding sites.

To explore this hypothesis, we infused small quantities of serotonin (5-HT) and other compounds (1×10^{-4} - 1×10^{-7}M) directly into the neostriatum of immobilized, locally anesthetized rats, while simultaneously recording single unit activity at the infusion site (within 0.2 mm) and 1.0 - 1.5 mm away. The 5-HT was infused at a rate of 0.01 µl/sec for 30 sec. Baseline activity, which ranged from 30 - 100 spikes/min, was recorded for at least 10 min prior to the infusion. Consistent with previous speculation, a local infusion of 5-HT decreased unit activity at the point of infusion, but produced a simultaneous increase in firing rate at the distant recording site. This disinhibition-of-surround pattern is suggestive of an interneuronal mechanism that allows for the relative enhancement of discrete neostriatal regions.

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276.15 AGE EFFECTS ON DOPAMINE AND NEURONAL DENSITY IN THE PORCINE CAUDATE NUCLEUS. L-F. Lue*, D. C. Beitz*, M. F. Rothschild* and D. D. Draper*. (Spon: R. C. McClure). Department of Animal Science and Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

Stress susceptible (SS) pigs exhibit locomotor disturbances characteristic of certain basal ganglia disorders. Because the biochemical and neuronal role in these disturbances is unknown in the pig, this study was conducted to determine whether dopamine concentrations and neuronal densities in the caudate nucleus were different in SS pigs than in stress resistant (SR) pigs. We observed that dopamine levels were markedly lower in the SS pigs than in the SR pigs in all age groups except in weanling pigs. There were significant age differences in neuronal density in both treatment groups with older pigs possessing fewer neurons per unit volume than young pigs. The decrease in neuronal density with age was greater in SS pigs than in SR pigs. All pigs used in this study were killed by electrical stunning and exsanguinated. To determine dopamine levels, approximately 60 caudate nuclei were collected from both SS and SR pigs. The caudate nuclei were frozen in liquid nitrogen and radioenzymatically assayed for dopamine. Dopamine levels were measured in five age groups in both SS and SR pigs. Average tissue dopamine concentrations (4,974 ng/g) in the caudate nuclei of SS pigs was significantly lower than that of SR pigs (6,635 ng/g). In both SS and SR pigs, there were marked age differences in dopamine concentrations with peak concentrations occurring at approximately 200 days of age and declining thereafter. The old SS pigs had a significantly greater decrease in dopamine levels than did the old SR pigs. Neuronal density in the caudate nucleus was determined from SS and SR pigs of three different ages (weanling, sexually mature, and aged). The caudate nuclei were fixed by immersion in 10% buffered neutral formalin, embedded in paraffin, serially sectioned at 7 µm, and stained with cresyl-echt violet. A cross section from the rostral, middle, and caudal regions of the caudate nucleus of each pig was systematically evaluated for neuronal density using a closed circuit video system. We found significant age differences in both SS and SR pigs with older pigs having fewer neurons per unit volume than young pigs. The decrease in neuronal density with age was significantly greater in SS pigs than in SR pigs. The dopamine and neuron density differences in SS pigs as compared to SR pigs support the concept that the caudate nucleus and dopamine may be involved in the porcine stress syndrome and that the stress pig may be a good model for studying basal ganglia disorders. (Supported by Biomedical Research Support Grant HHS 2 S07 RR07034-6).

276.16 DISTRIBUTION OF DYING NEURONS IN THE DEVELOPING CAUDATE NUCLEI OF NORMAL MICE. P.L. Mensah. Dept. of Anatomy, Univ. of So. Ca., Sch. of Med., 2025 Zonal Ave., Los Angeles, CA. 90033.

Previous work noted the occurrence of pyknotic neuronal nuclei in the developing caudate nucleus of the mouse (Mensah, Anat. & Embryol., In Press). This study was designed to generate data on the time course and distribution of these degenerating neurons. Pregnant C57Bl females were obtained from Simonsen Laboratories, date of birth of the litter being denoted day 0. Animals 1 hour to 14 days old were sexed using anogenital distance as an indication, sacrificed by nembutal overdose and perfused intracardially with a 2% glutaraldehyde-2% paraformaldehyde solution. Brains were weighed, divided into halves, and tissue chopped at 100 µm. The transverse extent of the caudate nucleus was dissected from each tissue section, dehydrated and flat-embedded in Epon-araldite in the tops of Beem capsules. One micron sections were cut from rostral (Level 2, Mensah, Brain Res., 1977) and caudal (Level 3) levels of the head of the nucleus. Plots of all pyknotic nuclei were made on camera lucida drawings of sections from a total of 96 tissue blocks. The data was tested for significance using a three-level nested analysis of variance.

The largest number of pyknotic nuclei occur for Level 2 at 3-4 days and for Level 3 at 5-6 days postnatally. However, these differences were not significant, most pyknotic nuclei occurring throughout the head of the postnatal caudate nucleus in the second half of the first week. Only at 1 to 2 days old is there a significant difference in the number of pyknotic nuclei occurring in the rostral vs. caudal portions of the nucleus ($p < .05$). In both males and females, pyknotic nuclei occurred randomly in the tissue sections at all time points. Of a total of 520 pyknotic nuclei studied, 55% were immediately adjacent to normal neurons and most were within cell clusters. Only 19% engaged in complex arrangements (cell pairs, cell triads) that are not seen in the adult nucleus. The neuronal nature of many of these cells was verified with the electron microscope. The occasional presence of monocytes in the lumen of blood vessels or penetrating the striatal neuropil was interpreted as an indication of the later phagocytosis and removal of these neurons from the tissue. By 14 postnatal days no pyknotic neurons were encountered in the tissue.

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276.17 POSTNATAL ONTOGENY OF CONNECTIVITY IN THE BASAL GANGLIA OF THE CAT: AFFERENTS OF THE SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA. A.M. Adinolfi, R.S. Fisher, C.D. Hull, M.S. Levine and N.A. Buchwald. Ment. Retardation Res. Center, UCLA, Los Angeles CA 90024.

The postnatal anatomical development of monosynaptic afferents to the substantia nigra (SN) and ventral tegmental area (VTA) of the cat was assessed with peroxidase histochemical techniques. In 6 neonatal kittens (<24 hr postnatal) and 2 adult cats, lectin-bound horseradish peroxidase (WG-HRP) was injected into the left ventral midbrain. The injection sites included most of the reticulate and compact zones of the SN and, to a variable extent, the VTA. After axonal uptake and retrograde transport of the marker, 4 distinctive groups of neuronal somata were labelled. The sites in which these neurons were found were similar in neonates and adults, and the labelled neuronal types found in each cell group were similar throughout postnatal life. 1) The striatal (S) inputs originated ipsilaterally from medium sized spherical neurons located in the caudate nucleus, putamen, nucleus accumbens, and olfactory tubercle. 2) The pallidal (P) inputs originated ipsilaterally from medium and large sized fusiform neurons scattered through the globus pallidus, substantia innominata, and lateral hypothalamic area. 3) The subthalamic (Sth) inputs originated ipsilaterally from medium sized spherical neurons. 4) The raphe (Ra) inputs arose bilaterally from medium and large fusiform neurons located in the dorsal mesencephalic raphe nucleus. The complements of labelled neurons in each cell group was S>P>Sth>Ra. Developmentally, the growth of the labelled neuronal somata in each group was Sth=Ra>P>S. While the density of the somatic labelling was light (S) or moderate (P, Sth, Ra) in each cell group regardless of age, it was greater in adults than neonates for each brain site. Labelled neurons were not evident in these brain sites when the labelled zones of the injection sites failed to impinge on tissue lying between the cerebral peduncle and the medial lemniscus (2 neonates, 3 adults).

These results indicate that the projection axons of neurons providing SN and VTA afferents come into existence prenatally. Each of the 4 cell groups projecting to the SN and VTA show some postnatal growth. Additionally, the developmental increase in the density of somatic labelling in each cell group suggests expansion of the terminal fields of these afferent cells.

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276.18 MORPHOMETRIC STUDY OF THE POSTNATAL GROWTH OF ENTOPEDUNCULAR NEURONS. C. Dvergsten*, C.D. Hull, A. Adinolfi, M.S. Levine, and N.A. Buchwald. Mental Retardation Research Center, UCLA, Los Angeles CA 90024.

Our laboratory is engaged in a morphometric analysis of the postnatal development of basal ganglia neurons. Previously, aspects of the development of medium-size spiny neurons in the caudate nucleus were described. The material reported in this abstract is derived from Golgi preparations of neurons in the inner shell of the globus pallidus(n. entopeduncularis (ENTO) in cats). Rapid Golgi impregnated neurons from 2-3 day old (N=2), 20-24 day old (N=2) and adult (N=1) cats were examined. Dendrites of well-impregnated cells were followed through 100μm serial sections for as many as 7 sections. The dendritic fields of 9 neurons in the 2-3 day old group, 8 neurons in the 20-24 day froup and 4 neurons in the adult cat were assessed with the aid of a computer-microscope system. Analysis of 3 parameters of dendritic fields indicates that most of the postnatal growth of ENTO neurons seems to occur during the first 20-24 days of life. The total dendritic length per neuron was 5638 ± 497μm at 2-3 days, 6592 ± 639μm at 20-24 days and 7030 ± 528μm in the adult. Mean dendritic field radius of all dendrites (Table 1,A) and mean length of all dendrites (Table 1,D) showed a similar trend.

Measurements of the longest dendrite of each cell, however, indicated a disproportionate growth increase both in dendritic radius (Table 1,B) and in total dendritic length (Table 1,E). The remaining dendrites, in contrast, increased their average radii to a much lesser extent (Table 1,C) and exhibited no discernible growth in total dendritic length (Table 1,F).

In caudate neurons the proportion of branches differ in the proximal and distal segments of the dendrite, while the branches of ENTO cells were evenly distributed along the dendrites at all three ages studied. The total dendritic length and radius of ENTO cells are at least twice those of the caudate cells.

TABLE 1

		2-3 day (9)	20-24 day (8)	Adult (4)
RADIUS OF DENDRITIC FIELD				
All dendrites	(A)	397 ± 24	434 ± 24	505 ± 34
Longest dendrite	(B)	493 ± 23	499 ± 33	720 ± 68
Remaining dendrites	(C)	362 ± 21	417 ± 28	448 ± 28
DENDRITIC LENGTH				
All dendrites	(D)	1282 ± 104	1449 ± 199	1491 ± 50
Longest dendrite	(E)	2242 ± 204	2386 ± 279	3012 ± 159
Remaining dendrites	(F)	1016 ± 103	1181 ± 166	1066 ± 69

Number of cells ( ); Values are means ± S.E.

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276.19 POSTNATAL ONTOGENY OF CONNECTIVITY INTHE BASAL GANGLIA OF THE CAT: OUTPUT NEURONS OF THE CAUDATE NUCLEUS. R.S. Fisher, M.S. Levine, C.D. Hull and N.A. Buchwald. Mental Retardation Research Center and Brain Research Institute, UCLA, Los Angeles, CA 90024.

The postnatal anatomical development of the output neurons of the caudate nucleus (Cd) of the cat was assessed with peroxidase histochemical techniques. In 6 neonatal kittens (<24 hr postnatal) and 2 adult cats, retrograde axonal transport of lectin-bound horseradish peroxidase (WG-HRP) injected into the substantia nigra (SN), was used to determine the neuronal origin of these fibers. Regardless of age, caudatonigral axons stemmed from medium-sized spherical neuronal somata that were labelled lightly. These cells showed little postnatal somatic growth. They were organized into cell clusters in both age groups, but were dispersed over larger tissue columes in adults. Anterograde axonal transport of WG-HRP injected into the head of the Cd in 7 neonates and 3 adults demonstrated labelled fibers that coursed caudally to form terminal fields in the ipsilateral pallidal segments and the SN in both age groups. These fields expanded with age as labelled elements became more dispersed and patchy.

Single Cd neurons in young kittens (8-50 days) and adult cats were marked by iontophoretic intracellular injection of HRP. Axonal morphology was delineated clearly in 10 kittens and 5 adult identified cells. All identified cells were medium-sized spiny Cd neurons which matched the somatic shapes, somatic areas and number of primary dendrites found in Cd somata labelled retrogradely after SN injections of WG-HRP. Regardless of age, identified Cd neurons had a single thicker projection axon which coursed toward the internal capsule. Multiple thinner collaterals branched and terminated locally in proximity to the marked dendritic field. The number and extent of the collaterals increased from 8-30 days. The characteristic evoked responses of Cd neurons to the activation of afferents (cortex, thalamus, and SN) shifted from simple excitations to excitation-inhibition sequences during this age period.

These results provide additional evidence to indicate that medium-sized spiny neurons are Cd output neurons. These neurons have both projection and local axons which develop at different rates. Their projection axons develop first, and extend into the pallidum and SN prenatally. As shown in our electrophysiological reports, these fibers provide functional monosynaptic caudatopallidal and caudatonigral inputs throughout postnatal ontogeny. In contrast, the local axon collaterals are elaborated largely during the early postnatal period. This slower outgrowth may be a factor underlying the late development of inhibition in the Cd.

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276.20 INTRINSIC GENERATION OF EVOKED INHIBITION IN THE CAUDATE NUCLEUS OF THE CAT. N.A. Buchwald, J.S. Schneider and C.D. Hull. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Hull et al. (Psychotrophic Drugs and Dysfunctions of the Basal Ganglia, NIMH Workshop 3: 92, 1969) and Buchwald et al. (Exp. Neurol. 38: 311, 1973) in studies of intracellular responses of caudate nucleus neurons, emphasized the fact that the great majority of these cells respond to stimulation of their monosynaptic afferents with an excitatory-inhibitory (E-I) sequence. In another report, Hull et al. (Exp. Neurol. 38: 324, 1973) speculated that the inhibitory component of the E-I sequence was probably a consequence of axon collateral firing of inhibitory caudate neurons upon neighboring cells. An alternate hypothesis might be that the apparent inhibition could be the result of an extranuclear input (e.g., it might be caused by an extrinsically initiated disfacilitatory process (Wilson, C.J. et al., Soc. Neurosci. Abst. 1: 849, 1981). While no crucial experiment has been mounted in this regard, two pieces of recent evidence bolster the belief that the inhibitory component of the E-I sequence is generated to a large extent, at least, by intranuclear events.

The heaviest inputs to the caudate nucleus come from the cerebral cortex and the intralaminar thalamus. Wilson, J.S. (Soc. Neusci. Abst. 1: 779, 1981) found that large cortical ablations, both made and tested acutely, and tested weeks after lesioning, failed to eliminate the inhibitory component of the E-I sequence, induced either by direct excitation of monosynaptic afferents or by sensory stimulation. In the present study, the possible role of the thalamus in producing inhibition in Cd neurons was studied. In 5 cats, acute lesions destroyed much of the thalamus including the central lateral, central median, parafascicular, dorsal median and ventral anterior nuclei. Subsequent to lesioning there was no demonstrable change in characteristics of either the excitatory or inhibitory potentials of the E-I sequence evoked in Cd cells by cortical or nigral stimulation (compared with pre-lesion results). This data therefore lends no support to the idea that the inhibitory component is primarily a consequence of externally induced disfacilitation. Further support to the notion of an intrinsically induced inhibition is derived from evidence (Morris et al., Br. Res. 173: 471, 1979) which indicates that the appearance of the inhibitory component of the E-I sequence is delayed in neonatal cats and the data presented at this meeting (Fisher et al.) indicating that the prominent perisomatic axonal collateral plexus found in adult caudate neurons is scanty or missing in physiologically and morphologically identified neonatal caudate cells.

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276.21 CONVERGENCE OF AFFERENT INPUT ON NEURONS OF THE VENTRAL ANTERIOR AND VENTRAL LATERAL THALAMIC NUCLEI IN CATS. R.A. Gazzara, N.A. Buchwald, C.D. Hull, R.S. Fisher, and M.S. Levine. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

We obtained intracellular neuronal records from 76 cells located in the ventral anterior and ventral lateral (VA-VL) thalamic complex of 21 locally anesthetized paralyzed cats. To determine the degree of convergence in these cells we tested their responsiveness to stimulation of their major monosynaptic and polysynaptic afferents: ipsilateral pericruciate cortex (Cx), caudate nucleus (Cd), globus pallidus-entopeduncular nucleus (GP-ENTO) and contralateral brachium conjunctivum (Cbl).

Antidromic responses to Cx stimulation were evoked in 25 cells with a mean latency of 4.6 msec. Most cells tested responded to stimulation of Cx (97%, 74/76 cells) or cerebellum (86%, 31/36 cells). Proportionately fewer neurons responded to Cd (76%, 52/68 cells) or GP-ENTO (49%, 27/55 cells). Most of these cells responded to Cx (97%), Cd (62%) and GP-ENTO (67%) stimulation with an initial inhibition, while the majority of cells responded to Cbl (55%) stimulation with an initial excitation followed by an inhibition. Seventy-eight percent (28/36) responded to stimulation of all three inputs indicating that a high degree of convergence of inputs occurs in VA-VL nuclei. These convergent cells were localized throughout both thalamic nuclei.

To date 5 cells in the VA-VL complex have been marked by intracellular injection of horseradish peroxidase for subsequent anatomical characterization. Four of these had small to medium-sized fusiform somata with 6-9 primary dendrites and one axon. The fifth cell was a medium-sized stellate cell with 11 primary dendrites and one axon. The four fusiform neurons responded to cortical, basal ganglia, and cerebellar stimulation while the stellate neuron only responded to cortical and basal ganglia but not to cerebellar stimulation.

In summary, a high percentage of VA-VL cells receive convergent inputs from cortex, cerebellum, and basal ganglia. These cells are located throughout VA and VL nuclei. Preliminary results with horseradish peroxidase marking indicate that the triply convergent cells are multi-dendritic neurons with fusiform somata.

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276.22 LONG-TERM BEHAVIORAL AND NEUROPHYSIOLOGICAL EFFECTS OF NEONATAL d-AMPHETAMINE ADMINISTRATION IN KITTENS. M.S. Levine, C.D. Hull, R.S. Fisher, and N.A. Buchwald. Mental Retardation Research Center and Brain Research Institute, UCLA, Los Angeles, CA 90024.

A short course of d-amphetamine administered to 4-6 month old cats produces a long-term (> 1 yr) decrease in the spontaneous activity of neurons in the caudate nucleus (Levine et al., Br. Res. 194: 263, 1980). The decrease in neuronal firing rate does not occur in cats in which neonatal lesions of the nigro-striatal path had prevented the normal accumulation of neostriatal dopamine. Because, in addition to this neurophysiological effect, administration of d-amphetamine results in persistent marked decreases in neostriatal dopamine, it seemed worthwhile to investigate the effect of administration of this agent to younger kittens in which dopamine concentrations had not reached adult values. Fifteen kittens (from 5 litters) were injected with 5mg/kg of d-amphetamine i.p. every other day from about 10 to 60 days of postnatal age. During this period normal caudate nucleus dopamine levels range from 10 to 60% of adult values. Five control animals (1 from each litter) were injected with saline on a similar schedule. The amphetamine-treated animals displayed two long-term effects. On the behavioral side they displayed hyperactivity in an open field situation and marked perseverative behavior in responding to a previously reinforced stimulus in a visual discriminative task. When the cats were between 1 and 2 years old an acute experiment was performed and the spontaneous firings of cortical and caudate units were measured. In the amphetamine-treated cats a marked slowing of caudate neuronal firing was observed (mean ISI $\pm$ S.E. = 3100 $\pm$ 477 msec in amphetamine treated cats vs 1449 $\pm$ 119 msec in saline controls, $p < 0.01$). Neurons in the precruciate cortex were apparently unaffected (mean ISI $\pm$ 1652 $\pm$ 303 msec in amphetamine cats vs 1271 $\pm$ 453 msec in saline controls). The results provide further evidence of long-term behavioral and neurophysiological consequences of amphetamine administration. Furthermore, these effects can occur when d-amphetamine is administered before dopamine concentrations have attained adult values.

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276.23 INTRACELLULAR CAUDATE NEURONAL RESPONSES FOLLOWING ACUTE ADMINISTRATION OF d-AMPHETAMINE. J.S. Schneider, M.S. Levine, S. Butcher, N.A. Buchwald, and C.D. Hull. Mental Retardation Research Center, UCLA, Los Angeles, CA. 90024.

This experiment was designed to test the effects of intravenously administered d-amphetamine on neuronal activity in the caudate nucleus. Changes in resting membrane potential and evoked PSPs were recorded intracellularly in a series of adult cats. Heart rate and blood pressure were monitored continuously during the experiments. Two doses of amphetamine; 0.1 mg/kg (N=4) and 0.5 mg/kg (N=17) were tested.

In amphetamine-treated animals as well as in saline-treated controls (N=7), responses evoked in the Cd neurons by stimulation of the cerebral cortex, substantia nigra, or the intralaminar thalamus always evoked an initial EPSP followed by an inhibition (E-I sequence). With the 0.1 mg/kg doses of amphetamine all cells showed long-term ($\leq$ 45 min) post injection increases in both the amplitude of excitatory and inhibitory components of the E-I sequence. These changes were more evident with cortical than with nigra or thalamic stimulation. In addition to increases in PSP amplitude the higher dosage of amphetamine produced another phenomenon in 57% of 23 cells; a depolarization beginning about 1 min after injection and ($\bar{X}$ latency = 71 sec; S.D.= 26) with an average amplitude of 17.7 mV (S.D. 12.5) and a mean duration of 172 sec (S.D. = 47.3) followed by a return to resting level. This effect was not blood pressure related since all cats responded to the high amphetamine dosage with large blood pressure increases while only half the cells showed the resting membrane depolarization and because the membrane change was not always coincident with the blood pressure increases. The independence of the amphetamine-induced changes in caudate neuronal activity and amphetamine-induced blood pressure increases was evidenced more clearly by the demonstration that quatenary amphetamine (which increases peripheral blood pressure but does not cross the blood-brain barrier) caused large blood pressure changes but no significant changes in evoked or resting membrane potentials (10 cells tested, 4 cats).

While a variety of factors may possibly have contributed to the outcomes of this experiment, we would single out the effects of amphetamine on dopamine release and its reuptake as a probable cause. The time course of the onset of the depolarization parallels that seen in experiments employing voltammetric measurements of striatal dopamine after amphetamine injection.

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277.1 THE SUPRASEGMENTAL ORIGIN OF THE PROJECTIONS TO THE CERVICAL SPINAL CORD IN POUCH-YOUNG OPOSSUMS (Didelphis virginiana). T. Cabana* and G.F. Martin (SPON: A.O. Humbertson, Jr.). Dept. Anat., Coll. Med., The Ohio State Univ., Columbus, OH 43210.

The opossum is born 12 days after conception and is available thereafter in an external pouch where it can be manipulated experimentally. We have reported previously on the origin of brainstem projections to the thoracic cord in a series of pouch-young opossums (Cabana and Martin, 1981) as shown by the retrograde transport of horseradish peroxidase (HRP). Thoracic levels were injected because of their accessibility. However, axons from several brainstem areas as well as from the cerebral cortex innervate the cervical but not the thoracic cord in the adult opossum.

In order to localize those areas which innervate the cervical cord at different stages of development, HRP and Nuclear Yellow were injected into cervical segments in a series of pouch-young opossums. Such injections resulted in neuronal labeling of those brainstem structures which also project to the thoracic cord. Those areas include: the raphe nuclei of the medulla and pons; the medullary and pontine reticular formation; the lateral, medial and inferior vestibular nuclei; the solitary complex; the dorsal column nuclei; the spinal trigeminal complex at all levels; the coeruleus complex; the red nucleus; the midbrain tegmentum and interstitial tegmental area; the interstitial nucleus of Cajal; the nucleus of Darkschewitsch and the hypothalamus. In most areas labeled neurons were more numerous after cervical than thoracic injections, except in the hypothalamus. Whereas projections from medullary sensory relay nuclei and the hypothalamus reach the thoracic cord relatively late (post-natal days 41 and 63 respectively), they are present in the cervical cord by at least post-natal day 28.

Other projections to the cervical cord take origin within the presumptive fastigial and posterior interpositus nuclei, intermediate and deep layers of the superior colliculus and from scattered neurons in and around the hypoglossal, facial and motor trigeminal nuclei and inferior olivary complex; they are established by at least post-natal day 28. Cortical projections to the cervical cord were first demonstrated with our methods at post-natal day 40 although they may be present somewhat earlier (Martin et al., 1980).

These data, together with those reported previously, emphasize the early growth of bulbar axons into the spinal cord in contrast to the relatively late arrival of axons from the motor sensory cortex. (Supported by BNS-80-08675.)

277.2 FURTHER STUDIES OF HOPPING RATS PRODUCED BY PRENATAL IRRADIATION. S. P. Hicks and C. J. D'Amato. Dept. of Pathology, Univ. of Michigan Medical Center, Ann Arbor, MI 48109.

Rats exposed to 150R of Xrays on the 13th, 14th, or 15th fetal day developed an unnatural hopping gait, defects of the central gray matter of the spinal cord and subcortical ectopias of the brain. The synchronous movements of paired hind and forelimbs was evident soon after birth and persisted through life except in some 15th-fetal-day irradiated rats who permanently recovered normal alternating gait of the forelimbs and rarely all four limbs, as juveniles. Hopping rats scratched synchronously with both hind limbs, but when jumping to a platform tilted 45° laterally, their downside limbs extended to keep a level posture. After surgical transection of the thoracic spinal cord, normal rats responded to stimuli with alternating extension and withdrawal of the hind limbs (mass reflex), but the responses of the limbs of hopping rats were synchronous, indicating that the principal site of the hopping disorder lay in the spinal cord. Nonetheless, the brain was able to make good use of the peculiar spinal locomotor generator (Hicks and D'Amato, Exp. Neur. 1980).

Present studies examine variation of the hopping movements of rats after 120R, 150R or 90R on the 14th fetal day, and the histologic characteristics of their spinal cords using dye stains and Stensaas and rapid Golgi methods. 120R rats resembled those exposed to 150R, but some switched back and forth between hopping and normal alternating walking gait. 105R rats differed qualitatively from 120R rats in slow motion movies and gait diagrams, switching back and forth between normal alternating walking and an abnormal gallop. Galloping, normally a running (80 cm/sec) gait, was at walking speeds and showed characteristics of hopping: hind feet were apposed side-by-side synchronously rather than placed asynchronously and not apposed. (Walking by 90R rats, still early juveniles, appears normal.) Histologically the spinal cords of 120R and 105R rats showed marked dorsoventral thickening of the gray commissure, as after 150R. Neuron types in this zone were typical of those in laminas VII and VI, but evidence of abnormal circuitry awaits further study. An unexpected finding in serial horizontal longitudinal Golgi sections of the cord in 120R and 105R rats was a lack of the normal longitudinal and circumferential bundling and braiding of dendrites of adjacent ventral horn motoneurons. Histogenic studies had suggested that a deficit or abnormal distribution of lamina VII interneurons might be found. But the abnormal configuration of the motoneurons, cells that originated earlier than most of their associated interneurons with whom they would be expected to interact developmentally, suggest that a more extensive cytoarchitectural malformation than was expected may be associated with the hopping disorder. (USPHS NS 10531)

277.3 DEVELOPMENT OF THE INTRALIMB LOCOMOTOR PROGRAM IN BULLFROG TADPOLES, Donald J. Stehouwer and Paul B. Farel. Dept. Physiol., Sch. Med., Univ. N. Carolina, Chapel Hill, N.C. 27514

We examined the development of neural coordination of muscular activity within an individual hindlimb using an in vitro preparation consisting of the CNS with the peripheral nerves to the hindlimb attached. All non-nervous somatic tissue was removed with the exception of the lumbar vertebrae and axial muscles through which the peripheral nerves passed. Dorsal roots were severed to eliminate sensory input. We recorded the activity of the following hindlimb nerves: cruralis, profundus anterior, and profundus posterior. The first two of these hindlimb nerves innervate muscles of the anterior thigh, whereas the third innervates muscles of the posterior thigh. Tadpoles ranging from Stage X to XVII (staging of Taylor and Kollros, 1946) were studied.

Patterned activity emerged in profundus posterior at least as early as Stage X, whereas patterned activity was not observed in nerves innervating anterior thigh muscles of the hindlimb until Stage XII. Beginning at Stage XII, the pattern of nervous activity was appropriate for repeated flexion and extension of the thigh. The development of the intralimb motor program, as indicated by the activity of the peripheral nerves innervating the thigh, coincides with the formation of the hindlimb musculature. The hindlimb muscles begin to differentiate at Stage VI, and attain their adult morphology by Stage XII (Dunlap, 1966). In the present study, we found an immature intralimb motor program at Stage X that apparently matured by Stage XII, as no further changes were detected during the remainder of larval development. Behavioral use of the hindlimb during locomotion can first be elicited between Stages XII and XIV (Stehouwer and Farel, in preparation).

We have previously described the electrophysiological development of the motor program responsible for interlimb coordination of "stepping" and "frog kicks" as recorded in vitro from the isolated CNS of the bullfrog larva. The interlimb motor program for stepping develops first, arising at least by Stage III, whereas that for frog-kicks does not emerge until about Stage XVII, the last premetamorphic stage. Thus, the interlimb motor program develops prior to differentiation of individual muscles of the thigh and before the intralimb motor program is manifested. Whether the concurrent development of the hindlimb muscles and intralimb motor program is coincidental or reflects a neural-muscular interaction is a question for future study.

277.4 ALTERED DISTRIBUTION OF MOTOR UNIT TYPES FOLLOWING SPINAL CORD TRANSECTION. S. Lofton*, J. Munson, G. Sypert and R. Foehring* (SPON: L. Willmore). Univ. of Fla. Coll. of Med., Gainesville, Fla. 32610.

Motor units of the cat's MG muscle were studied 2 weeks to 3 months following transection of the lumbar spinal cord. The following data were obtained in 15 acute experiments*:

	Motor Unit Type			
	FF	FI	FR	S
% of Population				
Transected (n)	61% (104)	7% (11)	8% (13)	24% (41)
Normal (n)	44% (116)	6% (16)	23% (60)	27% (70)
Rheobase (nA)				
Transected	21.2	13.8	11.9	5.2
Normal	21.5	15.4	11.8	4.8
Input Resistance (MΩ)				
Transected	.6	.7	1.0	1.8
Normal	.6	.7	.9	1.6
Conduction Velocity (m/s)				
Transected	94	87	91	77
Normal	97	107	99	84

*Methods and normal data from Refs. 1 and 2 and unpublished data. Underlined pairs of values differ $p < .05$. Twitch and tetanic contraction values were significantly reduced from normal.

The most striking effects are the 15% reduction in the number of FR motor units and the corresponding 17% increase in the number of FF motor units. This effect did not relate to the level of transection (L1 to L5). Similar alterations were observed in 4 other cats with dorsal hemisection of the lumbar spinal cord. Normal distributions of motor units and their properties were observed both immediately following and one week following transection.

Conceivably, many type FR motor units have altered both their mechanical and electrophysiological properties to convert to type FF motor units in response to one or another consequence of spinal cord transection.

References: 1) Fleshman, et al., J. Neurophysiol. 46; 2) Friedman, et al., ibid.

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277.5 ALTERED MICROSTIMULATION THRESHOLDS IN RAT MOTOR CORTEX FOLLOWING NEONATAL HEMICEREBELLECTOMY, D.L. O'Donoghue, G. Kartje-Tillotson, E.J. Neafsey and A.J. Castro. Dept. of Anatomy, Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153.

Microstimulation of the cerebral motor cortex has been shown to produce low threshold contralateral and higher threshold ipsilateral limb movements. This study examines the possible effects of neonatal hemicerebellectomy on these thresholds.

Under hypothermic anesthesia, 2 day old pups sustained right hemicerebellectomy by aspiration with glass pipettes. At maturity and while under ketamine anesthesia, the left motor cortex of these same animals as well as unoperated controls was explored by intracortical microstimulation (0.25msec pulses, 350Hz, 300msec train, 5-100 µamps) at a penetration depth of 1.7 mm. Evoked movements were noted according to laterality and stimulation threshold (i.e., current reliably evoking movement). Penetrations where stimulation elicited contralateral forelimb movements below 30 µamps and ipsilateral forelimb movements below 100 µamps were used for statistical analyses. Ten such points were mapped in each animal. Following stimulation, animals were sacrificed by anesthetic overdose and vascular perfusion with formalin; the brains were removed, photographed and inspected.

Both control (n=7) and experimental (n=3) groups demonstrated similar mean threshold currents for contralateral movements (18 and 16 µamps, respectively). In contrast, ipsilateral evoked movements in control animals had markedly higher stimulation thresholds (55 µamps) than did neonatally hemicerebellectomized animals (22 µamps). The mean difference between the ipsilateral and contralateral thresholds was found to be significantly different ($p<.005$) for the experimental as compared to the control animals. These data are believed to reflect the extensive neuroanatomical remodelling that occurs after neonatal cerebellar lesions and imply the involvement of cerebral cortex in compensation after cerebellar lesions.

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277.6 POSTNATAL DEVELOPMENT OF TRANSIENT CEREBRO-CEREBELLAR PROJECTIONS IN KITTENS. D. L. Tolbert* (SPON: L. C. Massopust). Murphy Neuroanatomy Research Laboratory, Depts. of Anat. and Surg. (Neurosurg.), St. Louis Univ., St. Louis, MO 63104.

Orthograde and retrograde intra-axonal labeling techniques were used to study the development of transient cerebro-cerebellar projections in kittens. Following ^{3}H-amino acid injections into the kitten somatosensory cortex labeled cerebro-cerebellar axons were observed in the superior and/or inferior cerebellar peduncles in animals up to 49 postnatal days of age. The growth of cerebro-cerebellar axons into the cerebellar peduncles occurred concomitantly with the innervation of rhombencephalic nuclei by incipient cortical axons. Many cerebro-cerebellar projections arise at the ponto-mesencephalic junction, descend in a corticotegmental tract and enter the ipsilateral superior cerebellar peduncle. The development of these projections paralleled the innervation of the ipsilateral pontine nuclei. Fewer cerebro-cerebellar axons pass through the contralateral superior cerebellar peduncle and arise directly from the pyramidal tract. These axons appeared slightly later than the ipsilaterally directed axons in the corticotegmental tract and at the time of innervation of the principle and the rostral spinal trigeminal nuclei. Cerebro-cerebellar projections through the superior cerebellar peduncles were directed primarily to the cerebellar nuclei and to a lesser extent to the cerebellar cortex. Concurrent with the innervation of more caudal rhombencephalic nuclei bundles of labeled cerebro-cerebellar axons projected from the pyramidal tract around the periphery of the brain stem and into the inferior cerebellar peduncles bilaterally. Cerebro-cerebellar projections through the inferior cerebellar peduncles were heaviest contralaterally and were strongly directed to the cerebellar cortex. In animals injected with both ^{3}H-leucine and proline and allowed to survive 4-7 days, the contralateral middle cerebellar peduncle was also labeled. The character of this labeling was strikingly different to the axonal labeling observed in the superior and inferior cerebellar peduncles and may have resulted from the release of labeled material into the pontine nuclei with subsequent labeling of periaxonal structures in the middle cerebellar peduncle.

Unilateral horseradish peroxidase injections limited to the cerebellum resulted in the retrograde labeling of layer V neurons in the cerebral cortex, thereby confirming the autoradiographic finding for direct cerebro-cerebellar projections. These HRP-positive neurons were localized rostrally in the cerebral hemisphere, were more numerous ipsilaterally than contralaterally, and were to a large extent clustered in anteroposterior oriented strips. (Supported in part by NIH Grant NS 15622.)

277.7 INCREASES IN THE EFFERENT NEURON POPULATION AND THE PROPORTION OF SLOW OXIDATIVE MUSCLE FIBERS IN THE RAT SOLEUS MUSCLE CORRELATE WITH BODY WEIGHT. R. L. Van Buskirk and W. D. Martin*. Departments of Physiology and Anatomy, West Virginia School of Osteopathic Medicine, Lewisburg, West Virginia, 24901.

In the rat, the proportion of slow oxidative fibers in soleus muscle increases as the animal ages. A previous study (Martin, 1980) demonstrated that this change was due to the stress of increased body weight rather than age per se. The current study was undertaken to determine whether corresponding changes occur in the motor neuron pool innervating soleus muscle.

Eight male Wistar rats weighing from 90 to 560 g were anesthetized using Nembutal. Soleus muscle was isolated and injected with 36 µl of 20% horseradish peroxidase using a microliter syringe and a glass micropipette with a 10µ tip diameter. In additional control animals denervation of neighboring muscles or direct immersion of the cut nerve to soleus are being investigated. Survival times varied from 24 to 72 hours. Each animal was perfused with normal saline and the triceps surae muscle was removed and frozen in liquid nitrogen. The animal was then perfused with 2.5% glutaraldehyde in phosphate buffer at pH 7.4, followed by 10% sucrose in phosphate buffer. The spinal cord was removed and stored in the sucrose solution at 4° C. Serial sections of spinal segments L3 to L6 were cut at a thickness of 50µ and reacted for HRP activity using tetramethylbenzidine (Mesulam, 1978). All sections were mounted and counterstained with neutral red. All labeled neuron profiles were counted using darkfield microscopy. Soleus muscle was serially sectioned at a thickness of 20µ and samples taken at intervals of 100µ. Adjacent sections were incubated for succinic dehydrogenase, or actomyosin ATPase after basic preincubation. The proportion of slow oxidative (SO) fibers and fast oxidative glycolytic fibers in the middle of the muscle belly was determined by random sampling. A direct correlation was noted between body weight and the proportion of SO fibers, and body weight and the number of labeled neurons after injection of HRP into soleus muscle. For example, a 90 g rat showed 250 HRP labeled neurons and 67% SO fibers; a 560 g rat showed 517 neurons and 95% SO fibers. Linear regression lines were calculated, and the correlation coefficient was .82 for neuron number, and .89 for the proportion of SO fibers.

These data demonstrate augmentation of the neuron pool innervating soleus muscle as the proportion of slow oxidative fibers increases in response to body weight. This implies a corresponding decrease in the motor unit size as the number of muscle fibers in soleus muscle remains constant.

277.8 Modification of Induced Descending Spinal Cord Activation of Motor Units in Man M.R. Dimitrijevic, J. Faganel*, A.M. Sherwood, and D.B. Vodusek*

Stimulation of the spinal cord via electrodes in the epidural space at stimulus levels slightly above sensory threshold has been shown to elicit muscle twitches through stimulation of posterior roots and descending pathways of the spinal cord. The muscle responses due to stimulation of the descending pathways usually appear with the lowest threshold in the short toe flexors, but can also be observed in other lower limb muscles, depending on the position of the electrodes, the strength of the stimulation and the functional condition of the spinal cord. The activity induced in the descending pathways can be modified by a variety of maneuvers. We have tested the nature of such modification in a series of patients who demonstrated such distant responses, and who had a variety of degrees of severity of paralysis in the legs. We found that subthreshold stimulation produces responses in muscle groups when the muscle groups are co-activated with a slight voluntary effort. Threshold or just suprathreshold stimulation produces responses which can be influenced by voluntary activation: responses in agonist muscles are facilitated and responses in antagonist muscles are suppressed. The same pattern of muscle twitch modification is observed upon elicitation of the Babinski response: facilitation of extensor muscles, and suppression in flexor muscles. Stimulation of the cutaneous receptors can modify the responses, plantar surface brushing increases the toe flexor response, and dorsal response decreases. Vibration also modifies the twitch responses.

The fact that some modification of muscle twitches could be observed even in muscles with absent volitional activity indicated that some features of supraspinal motor control were preserved.

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277.9 METABOLIC AND FATIGUE PROPERTIES OF SLOW AND FAST MUSCLE AFTER CHRONIC SPINALIZATION. K.M.Baldwin*, R.R.Roy, R.D.Sacks*, M.Short* and V.R.Edgerton.Kinesiology Dept. and BRI, UCLA, Los Angeles, CA 90024 and Physiology Dept., UCI, Irvine, CA 92717.

Two factors which regulate the metabolic properties and fatigue properties of muscle fiber types appear to be the quantity of activity and the genetic programs within the motor unit which operate independently of the activity level (Edgerton et al., Nature, 1980). To study the regulating factors further, normal adult cats and 6-12 month old cats with the spinal cord previously transected (T12-T13) at 2 wk of age were studied. The spinalized cats were subdivided into those that were exercised (TE) on a treadmill regularly (5days/wk;30 min/dy) and those that were not(TNE). After barbituate anesthesia the tendons of the soleus (S) and medial gastrocnemius (MG) were isolated distally along with the nerve supply. The vasculature was left intact. The nerve was stimulated for 2 min with trains of impulses lasting 330 ms of every second with a pulse frequency of 40 Hz. Following this procedure the muscles were prepared for biochemical analyses. To summarize the data below, the fatigue index (FI, initial tension/tension after 2 min) was unaffected by transection regardless of the presence or absence of treadmill exercise. Citrate synthase (CS) was unaffected in the S or MG by any treatment. Alpha-glycerophosphate dehydrogenase (α-GPD) of the MG was 49 and 41% greater in TNE and TE than in N and 18 and 62% in the S. These data lend support for some independence of FI and a mitochondrial marker enzyme to neuromuscular activity level. It also shows that changes in the glycolytic pathway are closely coordinated with changes in speed related properties (Roy et al., Soc. Neurosci., 1982). but are unrelated to fatigue properties of the muscle.

	CS		αGPD		FI	
	MG	S	MG	S	MG	S
N	14.22	23.83	56.11	19.00	36	97
	±1.51	±2.53	±3.62	±2.98	±6	±1
TE	11.28	27.50	83.40*	22.56	30	95
	±2.27	±4.94	±6.23	±2.77	±8	±3
TNE	11.82	30.44	79.22*	30.80*	31	93
	±0.46	±3.03	±4.43	±2.68	±8	±2

Mean S.E., *P<0.05 for TE vs N or TNE vs N

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277.10 CHRONIC SPINAL CORD TRANSECTION-INDUCED CHANGES IN CAT SOLEUS MOTOR UNITS. M. Fournier*, T.C. Cope and V.R. Edgerton (SPON: L.J. Goldberg). Dept. of Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Transection of the spinal cord induces changes in the properties of cat soleus (SOL) muscle (Buller et al., J. Physiol. 176, 1965) and motoneurons (Czeh et al., J. Physiol. 281, 1978). To better understand how motoneurons and muscle units interact under these conditions, both of these components were studied in single motor units (MU). SOL MU were studied because those which change after transection (Edgerton et al., Plasticity of Muscle, ed.: D. Pette, 1980) can be easily identified for this normally homogeneous muscle. Intracellular records were taken from SOL motoneurons in barbiturate-anesthetized normal (N; n=6) cats or in cats transected (T; n=5) at T_{12}-T_{13} at 2 wk of age 6-12 months previously. Mean after-hyperpolarization (AHP) duration and rheobase (I_{rh}) were significantly different between the 2 groups. Muscle units of T, activated by intracellular current injection, had shorter contraction time (CT) and lower maximum tetanic tension (P_o) and % of P_o produced at 20 pps (P_{20}/P_o) when compared to N. P_o per muscle weight (P_o/mw) was 53% less in T than N. Fatigue index (FI=tension after 2min/initial tension) was unchanged. Using CT and FI as criteria, the conversion of muscle units was from slow oxidative to fast oxidative glycolytic. This is supported by histochemical and biochemical indices (Roy et al. and Baldwin et al., Soc. Neuroscience, 1982). CT and AHP decreased in T relative to N such that a positive relation ($r=0.77$; $p < 0.001$) was maintained between these parameters (as for N alone) when data for both groups were combined. A negative correlation was found between P_o/mw and AHP in N ($r=0.67$; $p < 0.02$) and in T ($r=0.90$; $p < 0.01$). Although the linear regression shifted downward, the slope was similar for both groups with P_o/mw intercept for T being half that for N. These findings demonstrate that certain properties of both the neural and muscle components of MU are altered proportionately.

	CV (ms)	AHP (ms)	I_{rh} (nA)	R_{in} (MΩ)	CT (ms)	P_t (g)	P_o (g)	P_o/mw (g)	P_{20}/P_o	FI
N	74.3	120.7	2.5	3.0	64.9	2.76	15.90	5.7	0.92	0.96
	±2.9	±9.6	±0.4	±0.4	±2.4	±0.37	±1.70	±0.6	±0.01	±0.01
n=	20	16	16	7	29	27	18	18	16	13
T	75.6	58.7*	4.8*	2.7	31.4*	2.25	7.01*	2.7*	0.49*	0.93
	±2.2	±4.6	±0.9	±0.3	±0.9	±0.38	±1.41	±0.3	±0.07	±0.02
n=	19	12	12	3	21	21	11	11	11	9

Mean ± s.e.m.; n=number of units; CV=conduction velocity; R_{in}= input resistance; P_t=twitch tension; *=significantly different ($p < 0.05$).

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277.11 CONTRACTILE PROPERTIES OF FAST AND SLOW HINDLIMB MUSCLE FROM CHRONICALLY SPINALIZED EXERCISED AND NON-EXERCISED CATS. R.R. Roy, R.D. Sacks*, K.M. Baldwin*, M. Short* and V.R. Edgerton. Kinesiology Dept. and Brain Research Institute, UCLA, CA 90024 and Physiology Dept., UCI, Irvine, CA 92717.

The in situ isometric and isotonic contractile properties of the medial gastrocnemius (MG) and soleus (SOL) of low (T12-13) spinal cord transected (T) cats were determined. Kittens were cordotomized at 2 weeks of age and maintained for 6-12 months. Some T cats were exercised (TE) 30 min/day for 5 days/week on a treadmill. The muscles were tested at 36±1°C. There were no significant differences in any parameter between the TE and T non-exercised (TNE). Generally the SOL and MG were affected similarly by T for tension-related properties. There was 30-40% decrease in muscle weight (MW), maximum twitch (Pt) and tetanic (Po) tension in the MG and a 35-45% decrease in the SOL. Tension production per cross-sectional area (Po/CSA) was not affected in T cats in either muscle. Changes in biochemical assays of myosin ATPase activity (ATPase) and maximum velocity of shortening (Vmax, fiber lengths/sec) were closely related for both muscles: ATPase increased by 15% and Vmax 16-24% in the MG and by 60% and 40-55% in the SOL. Contraction time (CT) and ½ relaxation time (½RT) were shorter in the SOL of T in comparison to the normal cats while the MG of T were not affected. Chronic spinal transection had similar effects on tension of fast and slow muscle which was directly related to muscle cross-sectional area. However, increases in shortening velocities were larger in slow muscle and these changes were closely related with myosin ATPase activity.

	MW (g)	Pt (g)	Po (kg)	Po/CSA (kg/cm²)	CT (ms)	½RT (ms)	ATPase (μm/mg/min)	Vmax (fl/sec)
MG								
C	9.07	2273	10.2	2.03	45	19	.606	12.3
	±.42	223	.6	.09	1	3	.028	.4
TE	6.12*	1510	6.1*	2.15	47	29	.692	14.3
	±1.1	300	1.1	.12	3	4	.076	1.0
TNE	6.21*	1360*	7.2*	1.87	42	22	.701*	15.2
	±.52	190	1.2	.01	2	4	.036	1.2
SOL								
C	3.60	573	2.2	2.64	98	99	.233	4.7
	±.34	56	.2	.40	1	5	.049	.2
TE	2.11*	310*	1.2*	2.98	55*	41*	.379*	6.6*
	±.19	85	.2	.25	5	9	.083	.3
TNE	2.30*	311*	1.3*	2.42	58*	50*	.371*	7.3*
	±.51	67	.3	.48	2	4	.040	.8

Mean±S.E., *P<0.05 for TE vs N or TNE vs N

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277.12 NORMAL (N) MESENCHYMAL CELL IMPLANTS IMPROVE THE STRUCTURE AND FUNCTION OF DYSTROPHIC (D) MOUSE MUSCLES. P.K. Law. Departments of Neurology, Physiology/Biophysics, University of Tennessee, Memphis, TN 38163, USA.

A new technique is developed to facilitate uptake of N myoblasts into D muscles. Mesenchymal cells dissected from the limb-buds of day-12 N mouse embryos were transplanted into the right solei of 20-day-old N or D C57BL/6J-dy^{2J} mice. Contralateral, unoperated solei served as controls. The following results indicated that such transplantation improved the structure and function of the D test muscles.

Table 1. In vivo mechanophysiology at 37°C 6-7-months post-operatively.

	Normal(8)		Dystrophic(15)	
	Test	Control	Test	Control
Twitch tension (g)	3.6±0.3	3.0±0.5	2.9±0.7	2.1±0.5
Tetanus tension (g)	18.6±2.3	15.3±2.3	11.5±2.8	7.5±1.8
Wet weight (mg)	8.1±1.2	7.2±1.2*	6.1±1.2	4.5±1.0
Time to peak tension(msec)	15.9±2.9	15.3±1.9*	19.5±2.7	19.6±2.8*
Half-relaxation time(msec)	15.6±2.1	14.9±1.8*	24.2±6.2	27.3±7.6*

P<0.01 by paired-t except *, not significant. Mean ± SD given.

The test D muscle contained more fibers with high resting potentials than unoperated D control. Its mean resting membrane potential was intermediate between those of control N and D.

Table 2. Normal and abnormal appearing fibers 6-7-months post-operatively.

	Normal(6)		Dystrophic(6)	
	Test	Control	Test	Control
Normal	687.3±46.2	582.1±40.6	296.7±24.6	142.5±26.2
Abnormal	0	0 *	184.2±40.8	235.5±32.3
Total	687.3±46.2	582.1±40.6	480.0±37.3	378.8±54.0
% normal	100	100 *	61.92±6.26	37.62±2.65
% abnormal	0	0 *	38.08±6.26	62.38±2.65

Sham control solei that received similar surgical treatment but no myoblasts did not differ in mechanical and histological properties from the contralateral, unoperated solei. (Supported by MDA, and NSF PCM 7921008).

277.13 EFFECTS OF SHORT-TERM MONOCULAR DEPRIVATION UPON OCULOMOTOR FUNCTION IN THE RHESUS MONKEY. D.L. Sparks, M.R. Gurski* and T.L. Hickey, Department of Physiology and Biophysics, the Neurosciences Program, and Department of Physiological Optics, University of Alabama in Birmingham, Birmingham, AL 35294

Ongoing experiments are investigating the consequences of early visual deprivation upon the primate visual and oculomotor systems. This report describes the effects of short-term monocular deprivation (MD) (two weeks of lid closure begun at two weeks of age) upon visually-guided oculomotor tasks. Animals were tested after the lid-sutured eye had been opened for at least 18 months.

The horizontal and vertical positions of both eyes were recorded using the search coil technique (Fuchs and Robinson, 1966). Optokinetic responses were obtained by placing monkeys inside a cloth drum 90 cm in diameter. The drum, located within 6 ft. diameter magnetic fields, could be rotated in either direction at velocities up to 50 deg/sec. The interior of the drum consisted of alternating black and white vertical stripes, the width of which could be changed.

In long-term MD animals, rotation of the OKN drum in either direction (nasal/temporal or temporal/nasal) with stripes up to 15° in width failed to elicit OKN responses when vision was restricted to the deprived eye (Sparks, Mays and Hickey, 1982). In contrast, short-term MD animals, viewing the drum with the deprived eye did show OKN responses, but slow phase gain was reduced and directional asymmetries were observed. Normal OKN was observed when the nondeprived eye viewed the drum.

In the short-term animals, when both the deprived and nondeprived eyes are open, spontaneous saccades, OKN and VOR responses are conjugate. During attempts to fixate a visual target the horizontal position of the nondeprived eye remains stable while the deprived eye shows an initial slow adduction followed by further slow 'drifts' in horizontal eye position. The vertical position of both eyes remains relatively stable.

When visual input was restricted to the deprived eye, a conjugate vertical down-beat nystagmus was observed that continued during attempted fixation. Performance on a saccadic tracking task was impaired when the nondeprived eye was patched. When compared to performance with the nondeprived eye, saccadic latencies were increased, the peak velocity of many saccades was reduced and saccades to visual targets were less accurate.

In summary, two weeks of lid suture early in development produces severe deficits in the ability of the deprived eye to mediate visually-guided oculomotor responses. Recovery of oculomotor function following extensive training with the nondeprived eye patched is currently under investigation.

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277.14 STRIATAL NEUROCHEMICAL CHANGES FOLLOWING PERINATAL HYPOXIC-ISCHEMIC INJURY. M.V. Johnston. Neuroscience Lab., Depts. of Pediatrics and Neurology, and Cent. for Human Growth and Dev., Univ. of Michigan, Ann Arbor 48109.

A model of hypoxic-ischemic brain injury was used to study patterns of selective neuronal vulnerability in the immature rat. Unilateral common carotid artery ligation was performed in 7 day old pups under brief ether anesthesia. Following 2 hours recovery, pups were placed along with sham operated controls in a heated (37°C), humidified chamber containing 8% oxygen balance nitrogen for 2 hours. Carotid ligation alone had no discernible effects on the brain, but in combination with hypoxia it resulted in unilateral tissue injury (Rice et al, Ann Neurol 9: 131, 1981). Pups were sacrificed 2,3 or 6 weeks later for histological evaluation and assay of neurochemical markers for dopaminergic, GABAergic and cholinergic neurons in the striatum. The density and distribution of muscarinic cholinergic receptors was examined 2 weeks after the injury using in vitro quantitative autoradiography for ^{3}H-QNB on frozen coronal sections.

At the end of hypoxia, 83% of pups turned strongly toward the side of the ligation for up to 20 minutes (N=186). Two weeks after lesioning, 85% of ligated pups, but none of the shams had at least mild reduction in ipsilateral hemisphere mass; and 15% of these had severe damage with cortical scarring or cavitation. Striatal cell loss was more marked than damage to overlying cortex; and ipsilateral wet weight was 37+2% less than contralateral (N=50). Striatal tyrosine hydoxylase activity, ^{3}H-dopamine synaptosomal uptake and endogenous dopamine measured by HPLC-EC were similar in shams and injured pups. GABAergic markers, L-glutamate decarboxylase and ^{3}H-GABA uptake were also unchanged per mg tissue but reduced per striatum by 40%. In contrast, ipsilateral choline acetyltransferase activity and ^{3}H-choline synaptosomal uptake, presynaptic cholinergic markers, were reduced by 37% and 47% respectively (N=15, $p<.01$). Acetylcholineresterase histochemical staining also demonstrated decreased density in the ipsilateral, smaller striatum. Muscarinic receptors were 90% depleted in small regions of intense gliosis but were increased by 50% in the rest of the striatum. Examination of 4 and 6 week old pups suggested that early deficits are repaired since cholinergic markers returned to normal or supranormal levels with maturation.

Cholinergic neurons, which are among the last striatal components to mature, are more vulnerable to hypoxic-ischemic injury at one week than better differentiated GABAergic or dopaminergic neurons. Increased muscarinic receptor density and sprouting of remaining cholinergic axons may be important features of reorganization following this injury.

277.15 DEVELOPMENT OF SPINAL LOCOMOTION IN CATS. Grant A. Robinson*, Charles T. Leonard*, and Michael E. Goldberger. Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

It is well known that a cat will display hindlimb locomotion when its spinal cord has been transected at 12-14 days of age (Forssberg et al., 1980)[1] and this is commonly taken as evidence for the presence of a spinal pattern generator for locomotion. In contrast, cats transected as adults show poor,if any,locomotion. Thus, developmental changes in the spinal pattern generator for locomotion may be anticipated. We therefore examined the postnatal motor development of kittens transected at birth at T13 using behavioral tests. The following motor responses were tested and filmed daily in kittens transected within and the first 24 hrs after birth and in their normal littermates: proprioceptive placing, monopedal hopping, walking on a treadmill and walking overground (by displacing the body of the cat overground while the paws are in contact with a surface). Preliminary results show that after spinal shock wears off (24-36 hours) striking differences are seen in locomotion between spinal and control kittens. Treadmill walking (forward and backward), consisting of alternating flexion and extension of the hindlimbs,is first seen immediately after the disappearance of spinal shock. Extensor tone, however, is poor. In contrast, control kittens showed no similar locomotion until 3 weeks of age. Spinal kittens also showed a treadmill speed dependence for the type of hindlimb locomotion elicited; at speeds less than 0.05 m/s the spinal kittens walked, whereas at higher speeds they galloped. The normal kittens showed differential development of these two modes of locomotion. Even when they do begin to walk on treadmills the change of modes with increased speed is not seen. Furthermore, the control kittens showed no hindlimb monopedal hopping in the forward direction until 3 weeks of age, whereas the spinal kittens showed forward hopping soon after transection. In contrast, proprioceptive placing in the hindlimbs was seen in the control cats at birth and in transected kittens as soon as spinal shock had worn off. This placing was seen in forward, backward and lateral directions. The hindlimbs of the spinal animals showed poor support during locomotion until 2 weeks post transection. Controls did not develop such support until 3 weeks of age. Thus, locomotion in spinal kittens is initially superior to that seen in normals and appears to undergo more rapid early development. It therefore appears that descending influences are indeed present at birth and that the net effect exerted by them over the spinal generator for locomotion may be inhibitory at that time. Supported by NIH NS16629 and NSF BNS 241775.

[1]Forssberg, H., S. Grillner and J. Halbertsma, 1980. The locomotion of the low spinal cat. I and II. Acta Physiol. Scand. 108:269-296.

278.1 STIMULATION-INDUCED ACTIVATION OF CLAUSTRAL AFFERENTS TO ENTORHINAL CORTEX IN THE RABBIT. J.L. Bassett and T.W. Berger. Psychobiology Program, Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

In a previous study, cells in the dorsolateral and ventral regions of the rabbit claustrum were shown to give rise to fibers which terminate predominantly in the deeper layers of both medial and lateral regions of the entorhinal cortex (Berger et al., Soc. Neurosci. Abstr., 7, 1981). The present study used electrophysiological techniques to verify this projection and to identify functional characteristics of claustral-to-entorhinal connectivity in the New Zealand white rabbit.

Etched, insulated stainless steel electrodes having a tip impedance of 1-3 megohms (measured at 135 Hz in vitro), were used to record entorhinal cortical neuronal activity from layers V and VI. At several antero-posterior locations throughout the claustrum, insulated stainless steel bipolar stimulating electrodes were lowered in 0.5 mm steps (using an angular approach) beginning medial to the claustrum and moving progressively more lateral while observing the resultant evoked field potentials in entorhinal cortex. When stimulating electrodes were localized within the claustrum, a negative going potential having a latency to onset of 10-15 msec and a peak latency of 20-30 msec was consistently observed. This negativity was presumed to represent a current sink resulting from simultaneous activation of entorhinal neurons because action potential discharges were frequently associated with the negative-going wave. The onset of activation of entorhinal cortical cells ranged from 10-20 msec. A slight positivity was often seen preceeding the negative wave. As the recording electrode was lowered within entorhinal, the same negativity was observed in layers II and III, but reversed to a positivity in layer I. The positive going potential had the same peak latency (20-30 msec) as the deeper layer negativity. Thus, the layer I positivity represents a current source for the deeper layer negativity.

In total, these results verify the existence of claustral-entorhinal connections and indicate that claustral afferents are excitatory to entorhinal neurons. The neocortex has been shown to project extensively and in a highly topographic manner upon the claustrum (Carman et al., J. Neurol. Neurosurg. Psychiat., 27:46, 1964). Thus, the claustrum may function as an important source of neocortical input to the hippocampus, via the claustral-entorhinal projection described here. Consistent with this notion are the results of additional experiments within the present series showing that large-amplitude field potentials were also evoked within the dentate gyrus following electrical stimulation of the claustrum. Supported by The McKnight Foundation NSF grant BNS80-21395 and NIMH grant MH00343.

278.2 FUNCTIONAL CORRELATES OF THE SEPTAL AREA IN THE RAT: A 14C-2DG ANALYSIS. R. E. Watson, Jr., H.E. Siegel* and A. Siegel. Depts. of Physiology and Neurosciences, N.J. Medical School, Newark, N.J. 07103.

In an effort to develop a better understanding of the functional organization of the septal area, we employed ^{14}C-2-deoxyglucose (2DG) autoradiography in concert with electrical stimulation of different aspects of this structure in the anesthetized rat.

The experimental paradigm consisted of electrical stimulation delivered continuously for 30 sec. on and 30 sec. off for the period 5 min. preceeding and 45 min. following the injection of 2DG. Brains were then removed and processed for autoradiography.

The major findings were as follows: 1) lateral septal n. (anterior level): label was noted caudal to the level of stimulation and produced activation bilaterally within the lateral septal n. and posterior hippocampus and subicular cortex; 2) lateral septal n. (central level 1): label was followed from the stimulation site in the far lateral septum to the contralateral septal area, ipsilateral preoptic area and n. accumbens and to the ventral hippocampus and entorhinal cortex; 3) lateral septal n. (central level 2): label was followed from the stimulation site in the medial aspect of the lateral septal n. to the diagonal band, contralateral septum, n. accumbens, lateral preoptic region, all levels of hippocampal formation except its temporal one-third, ipsilateral entorhinal cortex, postcommissural fornix and mammillary bodies; 4) lateral septal n. (posterior level): increased optical density was observed rostral to the level of stimulation in the olfactory bulb, infralimbic cortex, n. accumbens, and diagonal band. Label was also followed to the contralateral septum and caudally to the preoptic area, bed n. of the stria terminalis, postcommissural fornix, bilaterally to the ventral two-thirds of hippocampal formation and to the basomedial amygdala: 5) medial septal n.: stimulation produced activation in the diagonal band, lateral preoptic area and lateral hypothalamus but not to hippocampus; 6) diagonal band nuclei: stimulation of any level of diagonal band resulted in the presence of activation in only the lateral preoptic area, lateral hypothalamus and infralimbic cortex but not in hippocampal formation; 7) septofimbrial n.: stimulation of this structure resulted in activation of the n. accumbens, infralimbic cortex, lateral septal n. on both sides, diagonal band nuclei, lateral preoptic area and hypothalamus, postcommissural fornix, anterior thalamic complex, stria medullaris and medial habenular n. as well as habenulo peduncular tract and interpeduncular n. The entire hippocampal formation with the exception of its anterior dorsal portion was intensely labeled, bilaterally.
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278.3 EFFECTS OF CORTICOSTERONE ON THE ELECTROPHYSIOLOGY OF CA1 PYRAMIDAL CELLS IN THE RAT HIPPOCAMPAL SLICE PREPARATION. C. T. Reiheld*, T. J. Teyler, and R. M. Vardaris. Neurobiology Prog., Northeastern Ohio Univ. Coll. of Med., Rootstown, Ohio 44226, and Psychology Dept., Kent State Univ., Kent, Ohio 44242.

It has been found that the rodent hippocampus binds corticosterone (CT) avidly, and this structure has been characterized as a target organ for CT. We investigated the changes in synaptic efficacy and transmission-time produced by three concentrations of CT: 4, 7, and 15 nM, corresponding to morning-resting, evening-resting, and stress levels respectively. Effects on amplitude and latency to peak of the CA1 field potential were assessed by obtaining stimulus-voltage IO functions prior to hormone administration as well as at 10 and 60 minutes post-hormone for each CT concentration.

There was a rapid response to 4 nM CT, such that field potential amplitudes were reliably increased relative to the control response at 10 minutes. The 7 nM and 15 nM concentrations also tended to be excitatory at 10 minutes although not significantly different from controls. Late effects were demonstrated for the 15 nM dose which depressed amplitudes compared to the controls at 60 minutes. Latency to peak voltage tended to be shorter at both the 7 and 15 nM concentrations.

It was concluded that, at resting levels, CT enhanced throughput in the Schaffer-collateral/CA1-pyramid synapse. In a concentration corresponding to stress levels, however, CT depressed amplitudes in this system. Concentrations of CT that attenuated the population response tended to shorten latencies. This pattern of results is quite consistent with observations of CT effects on cat spinal reflexes but not with a finding of biphasic changes in rate of single unit discharges in hippocampus of behaving rats. Possibly, in the latter study, concentrations near the stress level were achieved in the hippocampus, and response depression occurred at the longer post-administration test intervals.

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278.4 SPREADING DEPRESSION IN THE HIPPOCAMPUS: CHANGES IN EXTRACELLULAR $[K^+]$ AND $[Ca^{2+}]$. R. J. Reiffenstein, K. Krnjević, M. E. Morris and N. Ropert. Departments of Anaesthesia Research and Physiology, McGill University, Montreal, Canada H3G 1Y6.

In *IN VIVO* studies in the anaesthetized rat, double-barrelled liquid ion-exchanger microelectrodes were used to record field potentials and changes in $[K^+]_o$ and $[Ca^{2+}]_o$ in CA1/CA3 pyramidal cell layers of the hippocampus during paroxysmal spreading depression (SD), which was recorded infrequently and unpredictably during or following responses evoked by repetitive series of fimbrial or entorhinal stimulation (3-10 V, 0.1 ms, 5-100 Hz for 0.5-3 min). Observations in 7 different experiments (n = 13) were made at depths at or very close to the sites of the maximal +ve field potential. Negative tissue potential shifts, which were usually biphasic (with amplitude $\geq$15 mV, rise-time = 0.75(S.D.$\pm$ 0.32) mV/s, and duration = 65($\pm$15)s) occurred 10-110 s after the start of stimulation and were consistently followed by a +ve undershoot which lasted 1-3 min. They were associated with an ↑ in $[K^+]_o$ (from the resting level of ≈ 3 mM to a maximum of 28(S.D. $\pm$0.54, n = 9)mM, which was at times preceded by an earlier peak to ≈ 15 mM (n = 3). $[K^+]_o$ endured for ≈ 1 min and was followed by an undershoot for $\geq$5 min. At the same time or slightly later, $[Ca^{2+}]_o$ showed a large biphasic fall from a resting level of ≈1.3 mM to a minimum of 0.18(S.D.$\pm$0.13, n = 8), which was briefer than $\Delta[K^+]_o$ but was followed by a secondary long, shallow decline. Transient spike activity and a small -ve shift in tissue potential were frequently recorded before or at the onset of SD; a large +ve field potential - in response to fimbrial stimulation - was also briefly observed during its initial 10-20 s. These events were followed by absence of neuronal activity for ≈ 1 min, and a gradual recovery of the evoked +ve field. In one experiment a monophasic SD - which was not evoked by stimulation - occurred 7 min after an injection of 4 µg of kainic acid into the amygdala. The ionic shifts observed in the present experiments correspond in magnitude to those previously reported for the CNS; their biphasicity, however, appears to be peculiar to the hippocampus. The ease with which very small changes in stimulation (from $\leq$1 Hz to 3-4 Hz) can normally evoke large $\Delta[K^+]_o$ and $\Delta[Ca^{2+}]_o$ which resemble those evoked by much higher rates of stimulation in other brain regions *(Krnjević et al., Can. J. Physiol. Pharmacol. 58: 579, 1980; Krnjević et al., Can. J. Physiol. Pharmacol. 60: IN PRESS, 1982)* suggests that the hippocampus, with its powerful and finely balanced inhibitory and excitatory systems, may have a special susceptibility to SD. This in turn may be related to the neuronal degeneration and memory loss which have been observed following seizure activity and anoxia.

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278.5 PHASE RELATIONS OF HIPPOCAMPAL EVOKED POTENTIALS TO URETHANE-INDUCED THETA RHYTHM IN RATS. S.E. Fox and A.P. Rudell. Dept. of Physiol., Downstate Med. Ctr., Brooklyn, N.Y. 11203.

The theta rhythm recorded from the hippocampus of the rat during urethane anesthesia differs from that recorded during walking. In contrast to walking-induced theta rhythm, urethane-induced theta rhythm is lower in frequency, is abolished by atropine and has different phase relations to the firing of certain cell types. As part of a continuing effort to understand the synaptic mechanisms of theta rhythms this study was designed to determine the phase of urethane-induced theta rhythm at which hippocampal cells are most easily excited by stimulation of their afferents. Rats were anesthetized with urethane. Stimulating electrodes were implanted in entorhinal cortex (to monosynaptically excite granule cells) and in ventral hippocampal commissure (to monosynaptically excite pyramids). Recording electrodes were placed in the cell layers to record local EEG and evoked potentials. A fixed electrode was implanted in the dentate region to record a "reference theta". Stimuli were delivered at a rate of about one per four sec at randomly chosen phases of theta rhythm. Reference and local EEG preceding the stimulus, and the evoked potential following the stimulus were digitized. Data were collected for at least 512 stimuli at each recording site for each rat. The amplitude of the population-spike was cross-correlated with the EEG. This gave an estimate of the phase at which the amplitude of the population-spike was maximal.

Recordings were taken from 6 rats with good theta rhythm (r value for 2nd peak of autocorrelation > 0.5). For each of these rats at least one block of 128 trials showed a moderate correlation between the amplitude of the CA1 population-spike and the theta rhythm (r=0.3 to 0.7). The largest CA1 population-spikes occurred on the positive peaks of the reference theta. No dentate recordings were made from one rat. Only one of the remaining 5 rats showed a strong correlation between the amplitude of the dentate population-spike and the theta rhythm (r=0.5). In this case (as in CA1) the largest population-spikes occurred on the positive peaks of the reference theta. All of the other rats showed little or no relationship ($r < 0.25$ for all blocks of dentate data). There are two conclusions to be drawn from these data. First, the preferred phase for maximal population-spikes under urethane differed from the preferred phase during walking-induced theta rhythm by about 90°.[1] Secondly, in the dentate region it is much more difficult to demonstrate a relationship between excitability and phase of theta rhythm under urethane than it is in CA1, in contrast to the data from walking rats. (Supported by NIH grants NS17095 to S.E. Fox, NS14497 to J.B. Ranck and NS10987 to V.E. Amassian).

[1]Rudell, A.P., S.E. Fox & J.B. Ranck, Exp. Neurol.68:87 (1980).

278.6 LIMBIC EVOKED POTENTIALS AND BRAIN LEVELS OF TIN AFTER A SINGLE DOSE OF TRIMETHYLTIN IN THE RAT. Louis Zimmer*, Zuheir Hasan*, Dorothy Woolley*, and Louis Chang (SPON: R. Dagirmanjian). Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.

Trimethyltin (TMT) has been reported to cause lesions in restricted regions of the limbic system of the rat. The evoked potential technique was used to determine the relative effects and the sequence of effects of TMT on the neurophysiological functioning of various limbic system pathways. Adult Sprague Dawley female rats were implanted with bipolar stimulating/recording elctrodes in the prepyriform cortex (PPC), dentate gyrus (DG), CA3 of the hippocampus on both sides, and lateral septum. TMT chloride in a single 7.5 mg/kg dose in water was administered p.o. No changes in any evoked potentials were noted 24 hours after TMT, but some effects appeared at 48 hours. The evoked potentials which were most affected were those elicited in the DG by PPC stimulation, and these were greatly potentiated between 3-10 days after TMT with peak effect between 4-6 days. After 10 days the amplitude of the response declined markedly. The response in distal CA3 elicited either by activation of the mossy fiber or commissural inputs began to decline in amplitude 3 days after TMT and by 3 weeks was markedly depressed. By contrast the antidromic CA3 response evoked by stimulation of the lateral septum declined very little. Tentatively, this may be interpreted to mean that synaptic input to distal CA3 is affected more than are the CA3 pyramidal cells themselves. The response of DG to PPC stimulation indicated that TMT first activated, then depressed this system, either by an effect on the PPC or DG or on the entorhinal cortex, which is the relay station between the two areas. In order to determine whether the delay in onset of the effects of TMT on evoked potentials was due to a latent period in effect at the neuronal level or to a delay in penetration of TMT into the brain, flameless atomic absorption spectrometry was used to measure tin levels in whole brain and blood at 1, 2, 4, 10 and 20 days after TMT administration. Tin levels in brain were lowest at 24 hours, doubled at 48 hours, then doubled again at 4 days when peak levels were reached. Values were still relatively high at 10 and 20 days. Thus, the delay in onset of effects of TMT on evoked potentials may be related to the slow entry of tin into brain. Also, the time of peak effects on DG evoked potentials correlated with the time of peak levels of tin in brain. The prolonged retention of tin in both blood and brain may explain the continuing neuronal necrosis observed as late as 21 days after a single administration of TMT. (Supported by NIH grant ES-01503 to DW and fellowship ES-05145 to LZ. ZH was supported by a fellowship from the Royal Palace, Jordan.)

278.7 INTERACTION OF LOCUS COERULEUS AND LATERAL HYPOTHALAMUS UPON UNITARY ACTIVITY OF THE OLFACTORY BULB AND OLFACTORY TUBERCLE. R. Guevara-Aguilar, L.P. Solano Flores+, H.U. Aguilar-Baturoni, F.C. Barone and M.J. Wayner. Dep. de Fisiología, Facultad de Medicina, U.N.A.M. 04510-México, D.F. and Brain Research Lab., Syracuse University, 601 University Av., Syracuse, N.Y. 13210 USA.

In previous papers we have demonstrated influences from the mesencephalic reticular formation, lateral hypothalamus (LH) and locus coeruleus (LC) to the olfactory bulb (OB) and olfactory tubercle (OT). The aim of this work was to study the interaction between the LH and LC upon the OT and OB unitary activity. The experiments were conducted in Wistar rats of both sexes. The unitary activity was recorded with a micropipette 6-8 um of tip diameter. Simultaneously or separated single or train pulses were applied to the LH or LC. Stimulation frequencies between 20-80 Hz and current intensities of 100 uA to 1 mA were used. We found that the LH stimulation has a decreasing effect upon the OB unitary activity, longer suppressions with higher frequency stimulation. The stimulation with single pulses evoked a field potential with a 4 ms latency first component. In the other hand the LC stimulation has a suppression effect upon the OT unitary activity and LH stimulation produced both an increase or decrease effects. If LC stimulation preceded LH stimulation the suppression effect was not present on the OB. This effect is more evident on OT unitary activity and more complex responses were found. These results suggest a convergence of LH and LC fibres upon OB and OT neurons, being predominant the LC inhibitory effect.

278.8 THE ROLE OF THE MIDBRAIN AND HYPOTHALAMUS IN THE REGULATION OF NEURALLY ELICITED AGGRESSIVE BEHAVIOR AND FLIGHT IN THE CAT. M. Brutus, M.B. Shaikh*, H.E. Siegel*, G. Dooneief*, and A. Siegel. Dept. of Neurosciences, NJ Medical School, Newark, NJ 07103.

The purpose of this study was to identify sites within the hypothalamic-midbrain continuum of the cat that modulate aggressive and flight behavior elicited from the hypothalamus.

Initially, an electrode from which quiet biting attack (QBA), affective display (AD) or flight (F) could be elicited, was implanted into the hypothalamus. Then modulating electrodes were lowered at 1mm increments through the dorsal-ventral extent of both the hypothalamus and midbrain. The experimental design employed paired trials of single stimulation of the behavioral electrode and dual stimulation of both the behavioral and modulating electrode. The order of presentation of these paired trials followed an A-B-B-A sequence.

Modulating electrodes situated within the bed n. of the stria terminalis appeared to inhibit QBA, while in contrast, this same region and the adjacent anterior dorsolateral hypothalamus appeared to facilitate AD. Further caudally, the dorsal hypothalamus facilitated QBA, but had little effect upon AD. Stimulation of the posterior hypothalamic n. resulted in the facilitation of QBA and AD, while supramamillary stimulation facilitated AD but inhibited QBA.

Rostral midbrain facilitation of QBA was noted throughout much of the dorsomedial and dorsolateral tegmentum, while at more caudal levels, facilitation was limited to the lateral tegmentum. QBA inhibitory sites were noted in the medial aspects of the midbrain at caudal levels and throughout the rostro-caudal extent of the medial third of the ventral tegmental area. Concerning affective display, sites producing facilitation appeared to be widespread and were notable within the medial aspect of the tegmentum.

Flight was facilitated by modulating electrodes within ventral portions of the CM-PF complex, dorsomedial tegmentum and central gray, while sites in the anterior dorsal hypothalamus inhibited flight. Sites producing facilitation were prominent in the ventrolateral aspects of midbrain tegmentum, while dorsolateral regions tended to show inhibition.

The posterior hypothalamic and rostral midbrain areas generally had facilitatory effects upon QBA, AD and F, while other hypothalamic structures examined, appeared to modulate QBA and AD differentially. Our data tentatively suggests that the neural mechanisms controlling QBA are distinct from those regulating AD. (Supported by N.I.H. Grant NS 07941-13).

278.9 VOMERONASAL AMYGDALOID INPUT TO THE MEDIAL PREOPTIC "MATING CENTER" OF THE MALE GOLDEN HAMSTER. W.F. Maragos,* M.N. Lehman and S.S. Winans. Dept. Anat. & Cell Biol., Univ. Mich., Ann Arbor, MI 48109.

The medial preoptic area (MPOA) is an important androgen-concentrating brain area controlling male copulatory behavior in almost all vertebrates. In recent studies using the golden hamster, we have shown that vomeronasal and olfactory projections to the amygdala, particularly to the medial amygdaloid nucleus (M), are critical to male mating behavior in this species (Lehman et al., Science, 210:557). This evidence for the behavioral significance of M is consistent with autoradiographic data that, of the amygdaloid nuclei receiving chemosensory input, only M projects directly to the MPOA (Kevetter and Winans, J. Comp. Neurol., 197:81). In order to confirm this connection, and to examine the topographic organization of amygdaloid afferents to the MPOA, we iontophoretically applied horseradish peroxidase (HRP) (33% solution, Miles Laboratories) into several rostral-caudal levels of the MPOA in 36 anesthesized male hamsters. Two days later the animals were perfused and their brains processed according to the tetramethylbenzidine procedure for HRP histochemistry (deOlmos, Hardy and Heimer, J. Comp. Neurol., 181:213).

Histological analysis revealed that amygdaloid neuron cell bodies in M were labelled only after HRP applications in the caudal MPOA, in an area bounded rostrally by the crossing of the anterior commissure and caudally by the border of MPOA with the anterior hypothalamus immediately rostral to the suprachiasmatic nucleus. Following applications into this area, we observed labelled fibers in the dorsomedial quadrant of the stria terminalis, and traced these strial fibers to cell bodies confined to the dorsocaudal portion of M and throughout the rostral-caudal extent of the amygdalo-hippocampal area. In contrast, application sites confined either to portions of the MPOA rostral to the crossing of the anterior commissure, or to the anterior hypothalamus immediately caudal to the MPOA, produced no labelling in the stria terminalis or amygdala, although cell bodies in these brains were labelled in other forebrain areas at a distance from the application site.

These results confirm earlier observations describing the location of autoradiographically-labelled efferents from M to the MPOA (Kevetter and Winans, J. Comp. Neurol., 197:81) and provide new information about the restricted region within M from which these efferents arise. The terminal field of fibers projecting from M to the MPOA also coincides with the site of lesions within the MPOA which abolish mating behavior and impair chemoinvestigatory behavior in the male hamster (Bergondy, Winans and Powers, Neurosci. Abstr., this volume).

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278.10 COPULATORY AND CHEMOINVESTIGATORY DEFICITS FOLLOWING MEDIAL PREOPTIC AREA LESIONS IN MALE GOLDEN HAMSTERS. M.L. Bergondy*, S.S. Winans, and J.B. Powers. Dept. of Psychology, Vanderbilt Univ., Nashville, TN 37240 and Dept. of Anatomy, Univ. of Michigan, Ann Arbor, MI 48109.

The medial preoptic area (MPOA) in male vertebrates is a critical neuroendocrine substrate mediating masculine copulatory behavior (CB). MPOA lesions have eliminated or significantly reduced CB in all species studied. Although MPOA lesions in male hamsters have not been previously investigated, the critical importance of chemosensory stimulation has been well established. The vomeronasal and olfactory systems mediate this effect via specific projection pathways involving the medial nucleus of the amygdala (M) and the preoptic portion of the bed nucleus of the stria terminalis ($BNST_{po}$). Lesions of these regions can produce severe deficits in both CB and investigation of the female's anogenital region (AG). Whether the MPOA is also involved in this chemosensory pathway is unclear because disruption of the stria terminalis, which provides the only direct input from M to MPOA, has little effect on CB or AG.

To clarify the importance of the MPOA for male hamster CB and AG, we tested copulatory and chemoinvestigatory behavior after bilateral lesions aimed for the MPOA in 37 hamsters that had previously been castrated and administered silastic capsules of testosterone sc. CB tests lasted for 10 min or until the animal had ejaculated twice. Measures of CB included mounts, intromissions, and ejaculations (E) and measures of chemoinvestigation included the number of seconds investigating the female's anogenital region per minute of test (AG rate). Animals were given 2 pretests over 2 successive weeks and either 2 or 3 post-lesion tests on alternate weeks.

Eight males displayed severe CB deficits (no E's on any posttest), 13 showed partial deficits (fewer than 2 E's on all posttests), and 16 showed no deficits (2 E's on all posttests). Interestingly, post-lesion deficits in AG rates did not correlate with post-lesion CB deficits. Rather, both increases and decreases in AG rates were associated with all three CB deficit categories. This suggested that the MPOA lesions affecting AG and CB behaviors could be anatomically dissociated. Histological analysis of the lesions indicated that the behavioral deficits were the result of bilateral lesions placed precisely in the zone of termination of the fibers from M to MPOA and that lesions of the rostral-dorsal portion of this region resulted in severe CB deficits, while lesions more caudal, ventral, and medial to this region resulted in deficits in chemoinvestigatory behavior. The possible existence and functional significance of $BNST_{po}$ connections to these two zones of the MPOA remain to be clarified.

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278.11 PATTERNS OF SENSORIMOTOR AND TACTILE DISCRIMINATIVE DEFICITS FOLLOWING UNILATERAL LESIONS THAT PRODUCE "NEGLECT" IN RATS. G. D. Weese* and G. P. Frommer. Dept. Psychology, Indiana Univ., Bloomington, IN 47405.

Unilateral lesions in several brain sites product contralateral orientation and other sensorimotor deficits which have been labeled "neglect." We tested whether the pattern of these deficits is similar. We found that rats with unilateral lesions in lateral hypothalamus (LH) or globus pallidus (GP) displayed the same pattern of contralateral response deficits, which differed from patterns found in rats with lesions in substantia nigra (SN), ventrobasal thalamus (VB), or sensorimotor cortex (SM).

Albino rats were anesthetized, blinded, and implanted with electrodes in LH, GP, SN, or VB. These and other blinded rats (SM group) were trained in a small box with 1.3-cm-diam. holes on either side at which saccharin reward could be obtained (Hoyman et al., Physiol. Behav. 1979, 22, 139-147). The rats learned either to approach the hole on the same side as a mechanically delivered tactile stimulus or to approach the hole on the side opposite to the tactile stimulus. They also were tested for tactile orientation, open field turning, and limb reflexes. Then, unilateral lesions were made under anesthesia through the implanted electrodes or by aspiration for SM rats. After post operative testing, the lesions were verified from frozen sections. Only rats with accurately placed lesions are described.

Severe contralateral orientation deficits occurred in 8/10 LH rats, 5/7 GP rats, 3/6 SN rats, 2/5 VB rats, and 6/8 SM rats. Most LH and GP rats showed depressed contralateral and enhanced ipsilateral turning in the open field. Fewer SN, VB, and SM rats were depressed on this task, and depression was ipsilateral and contralateral equally. In the tactile discrimination, LH and GP rats showed severe deficits in all responding (correct, intertrial, error) contralateral to the lesion, regardless of the side to which the tactile stimulus was delivered. In contrast, responses ipsilateral to the lesion evoked by contralateral stimuli were unaffected. The SN, VB, and SM rats showed no deficits, milder deficits, or different patterns of deficits on this task. Some limb reflex deficits appeared in most rats from all groups.

These data show that different lesions that produce deficits in contralateral orienting do not produce the same pattern of deficits on other measures. They suggest that "neglect" describes only a certain level of behavioral analysis and that nigrostriatal or incidental somatosensory damage cannot fully account for the LH tactile neglect syndrome. Damage to a pallidofugal pathway may account for the very similar pattern of deficits following LH and GP lesions. (Supported by NIH Grant S05 RR 7031.)

278.12 PARALLELS IN INDIVIDUAL RESPONSE TENDENCY IN EATING EVOKED BY ELECTRICAL AND CHEMICAL STIMULATION OF HYPOTHALAMUS. S.E. Bachus & E.S. Valenstein. Psychology, Univ. Mich., Ann Arbor, MI 48109.

While we have argued that variability among rats in response to electrical stimulation of lateral hypothalamus (ESLH) primarily reflects characteristics of individual rats rather than electrode placement (Valenstein, Brain Behav. Evol. 2:295, 1969; Bachus & Valenstein, Physiol. Behav. 23:421, 1979), it has been assumed that differences in response to intra-hypothalamic norepinephrine (NE) are due to variable proximity of cannulae to paraventricular hypothalamus, presumed site of effective chemical stimulation.

To examine the relation between individual differences in behavior evoked by ESLH and NE, 24 adult male hooded rats received implanted bilateral electrodes aimed at LH and bilateral cannulae aimed at PVH. Each rat was tested twice with NE and once with saline through each cannula and twice with ESLH through each electrode. In NE tests, which were performed during the light half of the diurnal cycle to minimize spontaneous eating, rats were observed for 40 min. after infusion of 20 µg/1 µl/1 min. NE. During ESLH, 20 sec. trials were separated by 20 sec. inter-trial-intervals, and current was raised 1 µA each negative trial until 9 out of 10 consecutive positive trials were obtained which served as the ESLH data. Time engaged in eating Noyes pellets and Purina chow was recorded in both NE and ESLH tests. Consumption evoked through ipsilateral cannulae and electrodes was significantly correlated ($r=.50$, $p<.01$). Histological reconstruction of cannula and electrode positions confirmed that neither chemical nor electrical stimulation locus accounted for behavioral variability.

Several rats displayed a striking and parallel lateralization in response to both NE and ESLH. Asymmetry was calculated by subtracting right from left response for all rats. NE asymmetry was correlated with ESLH asymmetry across rats ($r=.51$, $p<.02$).

In response to ESLH, rats with access to a variety of objects often exhibit a consistent preference. Similarly, each of 6 naive rats tested 3 times with NE while exposed to water, chow, pellets, wood and liquid diet displayed a consistent preference across sessions bilaterally (1 chow, 3 pellets, 2 liquid diet). 12 naive rats were tested with multiple objects for NE and ESLH response. While preference was not always consistent between NE and ESLH, and spontaneous consumption of the very palatable liquid diet was high, combined duration of consumption of pellets, chow and wood was correlated between ipsilateral NE and ESLH ($r=.55$, $p<.01$). Asymmetry for NE and ESLH was again correlated ($r=.86$, $p<.01$).

Our results suggest that individual variability in duration of behaviors evoked electrically or chemically from hypothalamus reflects a common predisposition to respond to various forms of stimulation with consummatory behavior. The neural substrates mediating these behaviors appear to be similarly lateralized.

278.13 DIFFERENTIAL EFFECTS OF LIMBIC AND NON-LIMBIC ARCHISTRIATAL DAMAGE ON SCHEDULE-INDUCED ATTACK IN PIGEONS. Jeffrey D. Cross* and Irving J. Goodman. Department of Psychology, West Virginia Univ., Morgantown, WV 26506.

The avian archistriatum has been differentiated anatomically into limbic and non-limbic components. Functional analyses have supported this separation. The present study investigated rate changes in schedule-induced pecking attack behavior, a clearly quantifiable agonistic response, resulting from damage to one or another of these components.

Homogenous groups of pigeons were established on the basis of the frequency of pre-surgical attacks directed at a shielded stuffed target pigeon that was mechanized to move and vocalize. Following pre-surgical assessment and group assignment, surgically treated subjects received either a sham lesion consisting of bilateral brain puncture in the limbic (medial and posterior) or non-limbic (lateral and anterior) archistriatum or sustained electrolytic lesions bilaterally in one of these two areas. Following recovery from surgery, subjects were tested at 72 hr intervals on a variable time (VT-120 sec) schedule of food presentation in the presence of the shielded target pigeon. All groups, including unoperated controls, showed a decline in attack frequency as testing progressed. Those receiving limbic archistriatal lesions showed a significant decline in attack frequency for all test sessions following surgery, relative to controls and non-limbic lesioned animals. Non-limbic archistriatal subjects also showed a significant decline in attack rate compared to controls for the first few sessions following surgery. However, theirs was a transient decline, returning to normal over the last four sessions. This transient decline in attack rate may have resulted from a more generalized somatomotor deficit seen in these birds in the form of a transient impaired feeding ability, measured reductions in food intake and feeding time, also lasting only over the first few post-surgical test sessions. While limbic arcistriatal birds also showed the temporary feeding disruption, their attack rates never returned to normal. Thus it is not likely that the reduced attacks in limbic archistriatals is due to generalized somatomotor impairment.

Findings of this study provide further behavioral support for the structural and functional division of archistriatum into limbic and non-limbic divisions and the suppressive effects of limbic damage on aggressive behavior in birds.

278.14 NEURAL CIRCUITRY SUBSERVING AFFECTIVE DISPLAY ELICITED FROM THE FELINE HYPOTHALAMUS. S.A.G. Fuchs, A. Siegel, and H. Edinger. Depts. of Physiology and Neuroscience, UMDNJ, Newark, N.J. 07103.

Electrical stimulation of the medial hypothalamus elicited affective display behavior in cats which is characterized by mydriasis, piloerection, ear retraction, hissing, growling, and a paw-striking attack often directed towards a second cat. In one series of experiments, cats implanted with electrodes from which affective display had been consistently elicited were anesthetized and then injected with a ^{14}C-2-DG solution. Stimulation was delivered through the functional electrode in a regimen of 30 sec on 30 sec off for 5 min preceeding and 45 min following the injection. Brains prepared for x-ray autoradiography revealed that ventromedial hypothalamic stimulation produced activation in areas rostral to the stimulating electrode which included the anteromedial hypothalamus, medial preoptic area, diagonal band, bed nucleus of the stria terminalis, and lateral septal area. When stimulation was applied to the anteromedial hypothalamic sites which produced affective display responses, regional activation included the rostral sites as well as several caudal structures including the midbrain central gray and ventral tegmental area.

In a second series of experiments, a ^{3}H-leucine injection was placed into the region of the electrode tip from which the behavioral response was elicited in order to identify more precisely the pathways arising from neurons stimulated at that site. In general, the tritiated amino acid radioautographic study supported the ^{14}C-2-DG findings. Specifically, fibers arising from the region of the ventromedial hypothalamus (VMH) projected rostrally to the anteromedial hypothalamus (AMH) and neurons from AMH projected caudally into the midbrain tegmentum and central gray substance. The functional connections between ventromedial and anteromedial hypothalamic "display regions" were further examined in a third series of studies. Dual stimulation methods were employed to demonstrate that stimulation of affective display sites in the VMH could facilitate the occurrence of the response elicited from the AMH. Further, preliminary observations indicated that lesions placed within the AMH from which affective display had been elicited, served to elevate the latency and/or threshold for the occurrence of the behavioral response elicited from the VMH. Collectively, the data supports the notion that the circuits in the hypothalamus subserving affective display includes an ascending component from VMH to the rostral preoptico-hypothalamic zone and a descending component from this rostral zone which supplies the midbrain central gray and ventral tegmentum.

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279.1 PARTIAL COHERENCE ESTIMATES OF THE EEG, R. J. Morgan, C. C. Turbes, and G. T. Schneider*. Physiology and Biophysics, Colo. State Univ., Ft. Collins, CO 80523.

The coherence estimate evaluates functional relationships between continuous processes in the EEG and may show relationships between brain regions. The data from eleven cats were used in these analyses. Portions of these records were selected to calculate the coherence function estimates.

The coherence and partial coherence functions are defined by Bendat and Piersol (Random Data: Analysis and Measurement Procedures. New York: Wiley, 1971). For two random processes, x(t) and y(t), the coherence function will be 1 if x and y are identical and will be 0 if x and y are independent. The coherence function and estimates of it range between these two extremes. In the brain, cerebral cortical and subcortical nuclei may have monosynaptic or multisynaptic interconnections. Two or more brain regions may be interconnected and intereact in a neurophysiological function.

Placing lesions in one of the brain regions and analyzing the coherence spectra of the remaining brain areas helps in understanding these physiological interactions. This method could lead to radical changes in the whole physiological state responsible for generating common cortical and subcortical EEG activity. An alternate approach is the use of partial coherence estimates. Partial coherence implies first eliminating from each of two signals, that part which can be considered as being determined by, or predicted by, a third signal.

Some of the data from a number of cerebral cortical /subcortical combinations show: 1) Highest coherence--right amygdala and right temporal cortex, 2-6 Hz 97%, 8-17 Hz 92% , 34-45 Hz 65%; partial coherence showed right amygdala to be the common generator. 2) Highest coherence--left precruciate cortex and left temporal cortex, 2-6 Hz 96%, 8-17 Hz 92%, 35-45 Hz 90%; partial coherence showed left precruciate gyrus to be the common generator. 3) Highest coherence--left nucleus accumbens and left amygdala, 2-6 Hz 52%, 8-17 Hz, 50%, 35-45 Hz, 64%; partial coherence showed left nucleus accumbens to be the common generator.

279.2 VISUAL EVOKED POTENTIALS TO LIGHT FLASH IN CATS: IS A FRONTAL SINUS REFERENCE ELECTRODE TRULY INDIFFERENT? P.M. Saxton and J. Siegel. Institute for Neuroscience and School of Life and Health Sciences, University of Delaware, Newark, DE 19711.

In 7 Flaxedilized cats, visual cortex evoked potentials (EPs) to an intensity series of brief light flashes were recorded differentially using either a screw over the frontal sinus or an insect pin in the neck muscle as the reference electrode. At the same time EPs from the cornea (the electroretinogram or ERG) and frontal sinus were recorded differentially against the neck muscle electrode. The EP recorded from the frontal sinus screw was a miniature of the ERG recorded from the cornea, with the same latencies and shape as the ERG but only 10 to 30% of its amplitude. At a fast presentation rate (1/sec) the amplitude of the b-wave of the ERG, and consequently the frontal sinus potential, decreased with increasing intensity of light flash, whereas at a slow presentation rate (1/3 or 1/5 sec) the ERG and frontal sinus potential increased in response to all but the highest intensities.

The shape of the visual cortex EP was consistent whether recorded differentially against the frontal sinus screw or a muscle electrode, but the amplitudes of the early components were strongly affected by the potential at the frontal sinus. The P_1-N_1 component was significantly larger ($p<.001$) when the frontal sinus screw was used as a reference electrode at each of the presentation rates.

The presentation rate as well as the reference electrode had a consistent effect on the change in EP amplitude to an intensity series of light flashes. Cortical augmenting (positive slopes, i.e. increasing EP amplitudes to an increasing intensity series) was more pronounced at the slow presentation rate ($\bar{\lambda}$=9.2) than at the fast presentation rate ($\bar{\lambda}$=2.2), using an indifferent neck muscle electrode as a reference. The use of a frontal sinus reference increased the EP amplitude slopes at the slow presentation rate (from 9.2 to 18.3), while decreasing the EP amplitude slopes at the fast presentation rate (from 2.2 to -6.7), in accord with the rate dependent slopes of the frontal sinus potential itself. It is concluded that measures of cortical augmenting-reducing to light flash stimulation are biased toward a reducing trend at fast presentation rates and toward an augmenting trend at slow presentation rates if the reference electrode is a screw over the frontal sinus.

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279.3 NEUROMAGNETIC LOCALIZATION OF TWO TRANSIENT COMPONENTS OF THE VISUAL EVOKED RESPONSE. F. Richer, D.S. Barth and J. Beatty. Dept. of Psychology, University of California, Los Angeles. 90024

Transient visual evoked magnetic fields (VEFs) to patterned stimuli were examined in humans using a superconductive magnetometer and were compared to the corresponding evoked potentials (VEPs) to elucidate possible relationships between the underlying neural activity responsible for the two sets of measures. Four subjects viewed a series of flashes of a checkerboard pattern presented at one meter. VEF were obtained from an array of 28 points on the scalp above the occipital cortex and covering an area of 6X12 cm around the Oz derivation. The VEPs were recorded from the 12 most posterior derivations of the 10-20 system. Two components could be clearly identified in most VEF traces at latencies of 120 msec (C1m) and 180 msec (C2m) post-stimulus. Both components reversed polarity with the hemifield stimulated and also along the vertical axis of the measurement array 3 to 4 cm above the inion. The amplitude extrema of C1m were separated by a distance of 4 cm on the scalp and those of C2m by about 5.2 cm. These distances are consistent with the presence of current sources located 2.2 cm and 3.1 cm beneath the scalp for C1m and C2m respectively. Also, the amplitude of the N120 component of the VEP was larger for derivations orthogonal to the axis separating the extrema of C1m than for derivations parallel to that axis. This would be expected if the two components were obtained from the same dipolar current source. No polarity reversals were found in any of the VEPs however and no clear correspondence was found between any of the other electrical and magnetic components. Results indicate that the high spatial resolution achievable with the VEF can be used to help infer sources of VEP components as well as to provide localization of global neural activity.

279.4 INTRACORTICAL ANALYSIS OF THE AUDITORY EVOKED POTENTIAL IN THE MONKEY. Mitchell Steinschneider*, Joseph C. Arezzo and Herbert G. Vaughan, Jr. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

To interpret the physiological significance of cortical auditory evoked potentials (AEPs), it is necessary to delineate the active neural elements, their laminar locations and the distributions of extracellular currents. We have recorded the AEPs and multiple unit activity (MUA) to 80 dB SPL contralateral clicks at 150-400 um intervals within, above and below the auditory cortex of the monkey. One-dimensional current source density analyses were performed to identify the laminar patterns of extracellular currents. Recording sites were histologically located.

The earliest near-field response in koniocortex arises from activity in thalamocortical (TC) fibers. This component is a non-inverting positivity, has an onset latency of 4.5-5.0 msec and precedes the initial cortical response by about 3.5 msec. Current sinks coincident with the TC fiber component are maximal in L.III and L.IV. Associated MUA can be traced from L.III into the underlying white matter, supporting the component's axonal origin.

The initial cortical AEP component is a positivity with an onset latency of about 8.0 msec and a peak of 10.5-13.5 msec. It is followed by a negative deflection, a positivity peaking at about 25.0 msec and a later negativity. These waves invert across koniocortex, are recorded in the far-field and are associated with complex and spatially distinct extracellular current distributions. The initial positivity is coincident with current sinks maximal in lower L.III and extending throughout L.III and L.IV. The second positivity is associated with sinks restricted to L.IV, while the negativities are related to sinks in L.I and L.II. Additional sinks are present which do not contribute components to the far-field AEP. Most prominent are middle laminae sinks which overlap in time with the initial negative deflection.

In contrast to the widespread distribution of TC fiber MUA, unit activity associated with the initial cortical response is restricted to middle laminae. This distribution combined with the extracellular current patterns suggests that the initial cortical component is derived from activity in vertically oriented cells with soma in L.III and L.IV and apical dendrites in L.I and L.II. Current distributions for the second positivity are also consistent with a vertically oriented generator with dendrites in the superficial laminae. On the other hand, the closed-field generators suggest activity in stellate neurons.

Work is progressing to define the AEP generators in koniocortex at a greater spatial resolution, and to relate their current patterns with those in surrounding auditory fields.

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279.5 LATE POSITIVE EVENT-RELATED POTENTIALS IN CYNOMOLGUS MONKEYS (Macaca fasicularis). K.A. Paller,* S. Zola-Morgan,* L.R. Squire, and S.A. Hillyard. (SPON: N. Okudaira). Dept. of Neurosciences and Dept. of Psychiatry, UCSD Sch. of Med., La Jolla, CA 92093 and VA Medical Center, San Diego, CA 92161.

Unpredictable stimuli deviating from a repetitive sequence evoke late positive event-related potentials (ERPs) in human subjects under a variety of conditions. These long-latency waves (termed P300 or P3) have been linked with cognitive processes of stimulus evaluation, orienting, and expectancy, though their neural bases are largely unknown. We have been investigating whether stimilar ERPs can be recorded from cynomolgus monkeys.

Two untrained monkeys were presented with a Bernoulli sequence of two tones (90% of 1500 Hz and 10% of 600 Hz) while sitting in a primate chair. These tones (all 50 msec in duration and delivered at one-second intervals) had no conditioned significance for the animal. EEG was recorded from chronically-implanted skull screws and computer-averaged to generate ERPs for the frequent and infrequent tones. The latter elicited an enlarged positivity over frontal and central midline sites, extending from about 200 to 400 msec after the stimulus.

These same two tones were also presented in a Bernoulli sequence at equal probabilities to investigate the effect on the ERP of the tones immediately preceding the eliciting tone. When the eliciting tone was preceded by a short sequence of the other tones (BBBA or AAAB), the late positive ERP was larger than when the tones did not change (AAAA or BBBB). The overall relationship between ERP amplitude and the sequential ordering of stimuli resembled that seen for the P3 in humans.

In another condition, a monkey vocalization (100 msec growl-like sound) was interspersed at random (10%) in a sequence of high-pitched (80%) and low-pitched (10%) tones. The late positive ERP was largest to the vocalizations, intermediate to the rare tones, and smallest to the frequent tones. Although the ERP observed in untrained monkeys resembled the human P3 in waveform and in sensitivity to stimulus probability, further tests are necessary to establish a correspondence between species.

279.6 THE EFFECTS OF INTERSTIMULUS NOISE ON SHORT-LATENCY AUDITORY RESPONSES. T.A. Jones and J.M. Horowitz. Department of Animal Physiology, University of California, Davis CA. 95616.

In waking and anesthetized cats (3) and rats (3), wide-band noise was presented between auditory stimuli in order to examine the influence of interstimulus noise on auditory brainstem responses (ABRs). The effect produced by the noise at various intensity levels (0 to 40db relative to ambient noise) and durations (10 to 500 msec) was examined as a function of pip and/or click intensity, repetition rate and stimulus coupling (i.e. air or bone coupled auditory stimuli). Noise was presented monaurally (ipsilateral as well as contralateral to a monaural stimulus) and binaurally and was always air-coupled sound. Pip/click stimuli were presented both monaurally and binaurally and were presented either as air-coupled or bone-coupled stimuli. Interstimulus noise had significant and complex effects on the amplitude and latency of auditory potentials. While amplitudes were consistently reduced for positive peaks 2 and 4 , interpeak latencies shifted in various ways depending on noise and stimulus parameters. The magnitude of effects were dependent upon pip/click intensity as well as the intensity of the noise, where the magnitude of effects were inversely related to the stimulus intensity. Theoretically, both auditory fatigue and/or acoustic reflex mechanisms could be responsible for the changes in the responses. The relative contribution of each mechanism was examined in the present study.

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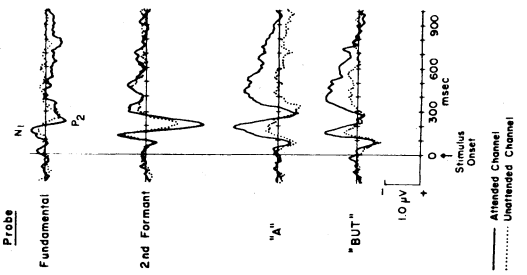
279.7 WHAT'S IN A SPEECH CHANNEL? D. L. Woods S. A. Hillyard and J. C. Hansen, Clinical Neurophysiology Laboratory, U.C. Davis, V.A. Medical Center, Martinez, CA 94553. and Dept. of Neurosci., UCSD, La Jolla, CA 92093.

At a cocktail party or in other noisy environments it is possible to attend to one speaker and ignore other voices. What features of the attended speaker's voice permit this selective processing?

To answer this question we examined event-related brain potentials (ERPs) elicited by probe stimuli mixed with channels of dichotically presented speech. The probe stimuli were either tones presented at the fundamental or mean second formant frequency of the speaker, or syllables spoken by the speaker which had been digitized and mixed with the speech by computer. Subjects either subvocally shadowed (repeated phrase-by-phrase) or carefully listened to one speech passage and ignored the other, as probe ERPs were simultaneously recorded from attended and non-attended passages.

Probe ERPs showed attention-related changes which varied as a function of the relation between the probe and the speaker's voice. The largest changes were observed for the syllable probes. The results suggest that selective linguistic processing depends upon a detailed acoustic representation of the speaker's voice.



279.8 A COMPARISON OF TOPOGRAPHIC AND VECTORGRAPHIC REPRESENTATIONS OF THE SCALP POTENTIAL DISTRIBUTION IN MAN. G. Goldberg*, H. C. Kwan, J. T. Murphy, Depts. of Physiology and Rehabilitation Medicine, University of Toronto, Toronto, ON. M5S 1A8

The temporal evolution of the scalp electric potential distribution may be represented by series of topographic maps or by generating frontal, sagittal and horizontal planar curves of the vector EEG using three-channel orthogonal lead recording (Tadahiko, I. et al: Arch Otorhinolaryngol 226:55, 1980). A software system has been developed on a color graphics desktop microcomputer for processing and display of multichannel EEG and evoked potential data. This system is capable of generating both types of graphic representations. To determine the relationship between these two graphic representations, data from a long-latency somatosensory evoked potential paradigm in six normal subjects was obtained using twenty monopolar leads at electrode sites in the International 10-20 system and three orthogonal bipolar leads--Fpz-Oz, T3-T4 and Cz-mentum. Correlation between the two representations was observed on subintervals of the analysis period suggesting that vectorgraphic representation may be a useful technique for analysis and display of the temporal evolution of the scalp potential distribution under specific circumstances.

Supported by the Medical Research Council of Canada

279.9 INTRACRANIAL RECORDINGS OF EVENT-RELATED POTENTIALS IN HUMANS ENGAGED IN COGNITIVE TASKS. G. McCarthy*, C.C. Wood, T. Allison, W.R. Goff, P.D. Williamson*, and D.D. Spencer*. V.A. Medical Center, West Haven, CT and Yale U. School of Medicine, New Haven, CT 06516

Event-related potentials were recorded simultaneously from scalp and chronically implanted depth electrodes in patients engaged in "oddball" categorization tasks. Auditory clicks, median nerve shocks, visually presented words, and stimulus omissions were used to elicit the ERPs in different blocks. Recordings were obtained from as many as 8 depth probes in patients undergoing evaluation for possible epilepsy surgery. There were four bilateral probe trajectories: Frontal probes were aimed at medial and orbital frontal cortex; supplemental probes were inserted above the forehead and aimed at the supplementary motor area; mid-temporal probes were inserted anterior to the precentral gyrus and were aimed at the anterior temporal lobe; and posterior temporal probes were inserted in the parieto-occipital area and were aimed at the hippocampus and amygdala. Each probe contained 18 equally spaced recording contacts from its point of insertion to its tip. ERPs were simultaneously recorded from 41 channels.

On the scalp, ERPs elicited by the task-relevant, low probability stimulus categories were marked by a positive potential (P300) with a latency of 300-700 msec and a posterior parietal distribution. Large amplitude potentials were observed at locations in the medial temporal lobe with similar latencies and morphologies to those at the scalp, with steep potential gradients and polarity inversions in the vicinity of the hippocampus and amygdala. These gradients, which were strikingly similar across stimulus modality, suggest that such medial temporal lobe activity is locally generated, in substantial agreement with the results of Halgren et al. (Science, 1980, 210: 803-805). These medial temporal lobe potentials exhibited sequential dependencies similar to those of scalp potentials and covaried in latency with scalp potentials across stimulus modality, suggesting that they could be the source of scalp P300. However, less consistently observed potentials at locations outside of the temporal lobe suggest the existence of other sources which could contribute to the scalp P300.

(Supported by the Veterans Administration and by NIMH Grant MH-05286).

279.10 ENDOGENOUS EVENT-RELATED POTENTIALS FOLLOWING TEMPORAL LOBE EXCISIONS IN HUMANS. C.C. Wood, G. McCarthy*, T. Allison, W.R. Goff, P.D. Williamson*, and D.D. Spencer*. VA Medical Center, West Haven, CT and Yale U. School of Medicine, New Haven CT 06516

Recordings of endogenous event-related potentials (ERPs) from chronically implanted depth electrodes in human patients have demonstrated locally generated activity in the vicinity of the hippocampus, amygdala, and other medial temporal lobe structures which appears to share many properties of endogenous ERPs recorded from the scalp (Halgren et al., Science, 1980, 210:803-805; Wood et al., Ann. N.Y. Acad. Sci., in press; McCarthy et al., this meeting). To determine whether temporal lobe structures are the sources of endogenous ERPs on the scalp, we investigated the effects of temporal lobe excisions on such potentials.

ERPs were recorded simultaneously from 41 scalp electrode locations in an 80%-20% "oddball" task using auditory, somatosensory, and visual stimuli in separate blocks of trials. Ten patients undergoing temporal lobe resection for relief of medically refractory epilepsy were studied, five postoperatively and five both preoperatively and postoperatively. Patients underwent resection of either: (a) the anterior temporal lobe including the amygdala and much of the hippocampus; or (b) the tip of the temporal lobe, amygdala, and hippocampus, sparing most of the lateral temporal surface. In normal subjects, ERPs recorded in an oddball task are dominated by a P300 component having a broad, bilaterally symmetric scalp distribution and a parietal amplitude maximum. If such a distribution were due to summated activity from bilateral temporal lobe sources, then unilateral temporal lobe excisions should abolish or greatly reduce activity on the side of the excision.

Following temporal lobe excisions, P300 was not markedly reduced on the side of excision; in fact it was even enhanced in some patients. These results are difficult to reconcile with the hypothesis that P300 on the scalp is generated exclusively by temporal lobe sources. The postoperative enhancement of activity on the side of excision in some patients could be due either to physiological effects of the excision on other sources or to altered conductive properties of the brain and skull following surgery.

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279.11 NEUROMAGNETIC LOCALIZATION OF INTERICTAL DISCHARGE IN PARTIAL COMPLEX EPILEPSY. D.S. Barth, W. Sutherling*, J. Beatty and J. Engel Jr. Dept. of Psychology, U.C.L.A. Los Angeles, Ca. 90024

The efficacy of surgical intervention in partial complex seizure disorders depends on the localization and quantification of focal cortical regions exhibiting ictal and interictal paroxysmal discharge in the EEG. We are currently exploring the potential of a new non-invasive technique, magnetoencephalography (MEG), as both an adjunctive localization procedure and research tool in partial complex epilepsy.

Four patients with partial complex epilepsy were referred for neuromagnetic measurement. Two displayed anterior temporal (1 and 2), one middle temporal (3), and one posterior temporal (4) interictal EEG spike discharges. Simultaneous 1 second averages were made of the MEG and six bipolar EEG leads over 10-15 consecutive EEG spikes for each of a series of closely (2 cm) MEG probe locations, forming a matrix on the scalp area covering the epileptic focus.

In two patients (1 and 3) the morphology and timing of the MEG spike was very close to that of the EEG and indicated superficial generators in the left anterior and right middle temporal cortex respectively, localized to within a 1 cm accuracy. Delayed spikes were also recorded in homologous contralateral regions of temporal cortex in both of these patients suggesting trans-commissural driving. The MEG spike of patient 2 was similar in morphology but had a shifting time relationship to the EEG that depended on scalp location. This, coupled with the large distance between magnetic poles suggested a slow spread of activity throughout a wider deep focus. Patient 4 presented a more complex magnetic picture suggesting multiple discharges in wide distribution over the posterior tempro-parietal region.

279.12 INCREASED CORTICAL EXTRACELLULAR POTASSIUM CONCENTRATION (K^+); CORRELATION WITH REVERSIBLE TRAUMATIC UNCONSCIOUSNESS. G. S. Sikand*, M. West*, H. G. Friesen* and V. Havlicek (SPON: K. Dakshinamurti). Dept. of Physiology, University of Manitoba, Winnipeg, Manitoba, R3E OW3, Canada.

Various studies subscribe to the theory of transient dysfunction of the reticular activating system as the cause of reversible coma following head injury. However, it was recently reported by us (West, 1980) that concussion of conscious animals produced significant depression of cortical EEG. This EEG depression could be reproduced by increased K^+ in the cortex. An increase in K^+ in the cortex and reticular formation (RF) following head injury was subsequently reported (Takahashi, et. al., 1981). The experiments reported here were designed to study the effects of increased K^+ in RF on cortical EEG. Sprague-Dawley rats (180 - 210 gm) were implanted with chronic bilateral epidural EEG electrodes. Cannulas were implanted in right half of brain stem RF. Days after surgery, 1 µl of 10% KCl solution was injected via cannula in conscious freely moving rats. EEG following KCl infusion was compared to EEG record prior to infusion. Initially following KCl infusion there was a slight EEG arousal pattern, beginning within 5-20 sec of infusion accompanied by tremors and contralateral hemiplegia. This arousal period which lasts for 2-6 min was followed by hypersynchrony of the EEG which was significantly different from both awake and arousal state ($p<.01$). The hypersynchrony which lasted for 12-20 min affected all frequency bands but was markedly increased for theta and delta ($p <.01$). This study clearly demonstrates that the EEG picture following increased K^+ in RF does not reproduce the EEG following concussion. These results, along with our previous observations, suggest that increase in K^+ in the cortex but not brain stem produces the EEG depression witnessed after experimental trauma to the head.

279.13 COHERENCE SPECTRAL ANALYSIS OF THE EEG DURING THE ACTION OF BARBITURATES. C. C. Turbes, G. T. Schneider* and R. J. Morgan. Anatomy Dept., Creighton Univ. Sch. of Med., Omaha, NE 68178.

During awake, conscious states, different regions of the brain show differences in the electrical activity. The action of the barbiturates tend to "iron out" these regional differences. With increasing blood levels, the barbiturate shows increasing suppression and increased, slower electrical activity. With higher blood levels, the electrical activity becomes slower and decreases in amplitude to an isoelectric level. In these studies, we are interested in the changes in the coherence and partial coherence functions that related to the changes in the EEG under the influence of the barbiturates.

The data from 34 cats are used in these experiments. Recordings are made using a polygraph and an FM tape recorder utilizing hardwire and telemetry procedures. Selected portions of analog records are sampled and analog to digitally converted. The Fast Fourier Transform algorithm is done to process in the frequency domain. Recordings are made before barbiturates are given as well as following 32 mg/kg, 64 mg/kg and 96 mg/kg doses.

In order to compare phase relations between two areas of the brain, two channels x(t) and y(t), use is often made of the coherence functions. The coherence function is frequency dependent since we can define only a phase value for a particular frequency component of the EEG. The coherence function is defined in terms of spectral density functions.

Cerebral cortical and subcortical nuclei are interconnected. These may be simple or complex multisynaptic connections. Two or more brain regions may be interconnected and interact in behavioral and physiological functions. How barbiturates act on these circuits is still speculative. We chose to use coherence and partial coherence anlaysis in these studies. Computing partial coherence implies first eliminating from each of two signals, that part which can be considered as being determined by, or predictable, on the basis of a third signal. These are data where barbiturates act on cerebral and subcortical circuits. With increasing barbiturate blood levels, there is generally a decrease in coherence of the high frequencies of 20 Hz to 100 Hz; this may vary in certain brain regions. There is a persistence or an increase in coherence at frequencies of 1 Hz to 20 Hz. There is a shift from the higher frequencies to the low frequencies at cortical levels at the lower levels of barbiturate concentration. The subcortical region maintains higher frequencies at higher barbiturate concentrations than do cortical areas. The changes in partial coherence are more complex since there is a relationship to the spatial origin of the EEG signals. These vary with the brain areas recorded and the rate of change of the barbiturate concentration.

279.14 SOMATOSENSORY EVOKED POTENTIALS AND SPINAL CORD TRAUMA IN PERFLUOROCARBON TRANSFUSED RATS. Henry F. Martin III, J.G. Blackburn*, S. Katz, and S. Trojanowski*. Department of Physiology, The Medical University of South Carolina, Charleston, S.C. 29425.

Vascular factors have been implicated as contributors to the failure of spinal cord conduction and loss of somatosensory evoked potentials (SEPs) following spinal cord injury. The following rat model is being developed to help assess which of the vascular factors may play an important role. By transfusing rats with a perfluorocarbon emulsion (blood substitute), oxygen can be carried to the tissues while removal of blood elements can alter thrombotic changes at the site of injury.

Adult rats (250-450g) were anesthetized by a combination of Ketamine hydrochloride (60 mg/kg - I.M.) and pentobarbital sodium (21 mg/kg - I.P.). The trachea was intubated, one carotid artery cannulated for blood pressure recording and blood withdrawl, and a femoral vein cannulated for infusions. The sciatic nerves were isolated for electrical stimulation and extradural screw electrodes were placed in the skull over the somatosensory receiving area. Control recordings of EEG, arterial pressure, and SEPs were made after surgery. The animal was then exchange transfused with a perfluorocarbon (FC-43) emulsion (Oxypherol - Alpha Therapeutic Corp.). Blood was withdrawn from the carotid artery and replaced by equal volumes of Oxypherol through the femoral vein. The process was continued until the hematocrit was less than 2%. The animals were respired with 95% O_2 / 5% CO_2. Animals were maintained for 2-3 hours after transfusion. Normal patterns of EEG and SEPs were recorded after transfusion. Trauma was produced by calibrated weight drop on mid-thoracic spinal cord exposed by laminectomy. Results will be presented comparing SEP changes after trauma in both control animals and perfluorocarbon transfused animals.

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279.15 USE OF EVOKED POTENTIALS TO ASSESS BRAIN DAMAGE AND RECOVERY IN SEVERELY MALNOURISHED INFANTS. I. Weiss, A. Barnet, Children's Hospital, National Medical Center, Washington, DC 20010, J. Flinn* George Mason University, Fairfax, VA 22030.

Cortical auditory and visual evoked potentials (AEPs and VEPs) were recorded from 25 infants when they were initially hospitalized for marasmus and from 19 of these infants at discharge. EPs were also recorded from 49 healthy infants. Control groups were selected to match the malnourished infants for sex and for age at both admission and discharge. The average age of the malnourished infants was 176 days at admission and 350 days at discharge.

A Fourier transform was performed on the data to give the frequency content of the EPs. At admission the AEPs of the malnourished infants had significantly lower amplitudes in the 12-24 Hz frequency range than those of the control group. A reduction in high frequency amplitude has previously been found to be characteristic of learning disabled and low IQ children. The VEPs of the malnourished infants had significantly smaller values of $d\phi/df$ (the slope of the phase versus frequency plot) in the 0-12 Hz frequency range. Visual inspection of the VEPs showed that the VEPs of the control infants last longer than those of the malnourished infants which is consistent with the larger values of $d\phi/df$. These two AEP and VEP parameters can be used to generate a discriminant function which distinguished between the EPs of the control and malnourished infants at admission ($p < .01$).

Paired t-tests showed that for the malnourished infants both the amplitude of the AEPs, in the range 12-24 Hz, and the 0-12 Hz value of $d\phi/df$ for the VEPs increased significantly between admission and discharge. Preliminary analysis indicates that there is no significant difference between the EPs of the previously malnourished infants at discharge and their age matched controls. These results show that frequency characteristics of the AEP and VEP reflect abnormal brain functioning due to malnutrition and the improvement in brain functioning due to hospitalization.

279.16 TOPOGRAPHY OF P300 AND EEG SPECTRAL POWER DENSITY IN SCHIZOPHRENIA. R. Morstyn*, F.H. Duffy and R.W. McCarley (SPON: J.L. Burchfiel). Dept. of Psychiatry and Neurology, Harvard University Medical School, Boston, MA 02115.

Two studies of the topographic distribution of brain electrical activity in schizophrenia were conducted. The first involved recording the spatio-temporal development of the P300 component in a group (n=10) of medicated chronic schizophrenics and a group of matched controls. A 'odd-ball' auditory paradigm was used (ratio frequent:infrequent=6:1), to elicit and isolate the P300 wave. Data was recorded from all regions of the scalp according to the 10-20 system and combined into a series of numerical (64x 64 point) matrices according to the method of Brain Electrical Activity Mapping (BEAM). Each matrix was displayed as a color image representing 4msec of evoked potential activity, and a series of images were sequentially displayed to reveal the spatial development of the P300 wave. By combining data from the two groups of subjects group-average P300 waves were able to be compared. Whereas the control group-average P300 showed the expected symmetrical development with a maximum in the centro-parietal area, the schizophrenics group's P300 was displaced anteriorly and to the right. Using the technique of Significance Probability Mapping (SPM) it was demonstrated that the maximum significant difference between the groups was localized to the left temporal region where the schizophrenic group was deficient in activity. In the second part of the study, EEG data were recorded in resting and specific conditions designed to activate different regions of the brain in a group (n=10) of medicated chronic schizophrenics, a group (n=5) of partially remitted unmedicated schizophrenics, and matched control groups. Spectral power density was computed using the Fast Fourier Transform and numerical matrices representing the topographic distribution of spectral power in a specified band and condition were constructed and displayed as color images. Using the SPM technique as well as other techniques to identify regions of maximal group separation it was found that the schizophrenic group showed increased low frequency power (0-7Hz) in the frontal regions and increased fast activity (12-32Hz) in post-central areas and the left anterior temporal region. The partially remitted unmedicated group appeared to show similar changes to the chronic group in the fast frequencies but did not show the frontal slowing.

280.1 SOMATOSTATIN RECEPTORS IN RAT PITUITARY AND CEREBRAL CORTEX. EFFECT OF GUANINE NUCLEOTIDES. A. ENJALBERT*, C. CHU*, E. MOYSE*, C. KORDON and J. EPELBAUM*. Unité de neuroendocrinologie. Centre Paul Broca de l'INSERM Paris 75014. France.

High concentrations of somatostatin (SRIF) are found in various brain areas where the peptide has been postulated to act as a neurotransmitter. However its mechanism of action at the membrane level is still unclear, even on adenohypophyseal cells where it could act through an inhibition of the adenylate cyclase. In the present work, we compared bindind sites to ^{125}I N Tyr-SRIF to membrane preparations from rat cerebral cortex and adenohypophysis and assessed the effect of guanine nucleotides on the binding.

Tissues were homogenised in 0.05 M Tris Hcl buffer, pH 7.5. Nuclei were discarded and membrane prepared by differential centrifugation. ^{125}I N Tyr-SRIF was used as ligand. Degradation of the ligand in the presence of the membrane preparation was prevented by adding bacitracine (0.1 %) in the medium. Under these conditions, ^{125}I N Tyr-SRIF binds with high affinity to one class of sites on membranes prepared from anterior pituitary (Kd = 0.91 nM, binding capacity = 104 fMoles/mg protein) and cerebral cortex (Kd = 0.80 nM, binding capacity = 53 fMoles/mg protein). In both structures, ^{125}I N Tyr-SRIF binding is specific, since it is displaced in a dose dependent manner by native SRIF (Ki = 0.10 ± 0.05 nM for pituitary and Ki = 0.23 ± 0.04 nM for cortical membranes) but not by neurotensin, bombesin, substance P, vasoactive intestinal peptide, LH-RH, TRH and D ala$_2$ Met-Enkephalin, tested at 1 µM.

Moreover we found that guanine nucleotides are capable of decreasing ^{125}I N Tyr-SRIF binding to pituitary and cortex membranes with an EC_{50} of about 10 µM. Both GTP and its non-metabolized analog Gpp(NH)p are effective, whereas GDP is less active and GMP or cyclic GMP are inactive. This effect appears limited to guanine nucleotides since ATP, ADP, AMP and cyclic AMP are ineffective. In order to evaluate the kinetic mechanisms involved in guanine nucleotide effects, we measured association and dissociation rates of ^{125}I N Tyr-SRIF binding. Dissociation was markedly accelerated in both structures in presence of Gpp(NH)p (50 µM).

In summary our data demonstrate that SRIF binding sites in brain and pituitary exhibit similar kinetics and are modulated in the same way by guanine nucleotides. They suggest that somatostatin receptors in brain as well as in pituitary may be coupled with adenylate cyclase. Some central effects of the peptide could thus be mediated by cyclic AMP.

280.2 EFFECTS OF FOOD DEPRIVATION ON CHOLECYSTOKININ RECEPTOR BINDING IN RAT BRAIN. J.A. Finkelstein, A.W. Steggles*, P. Martinez* and M. Praissman*. Northeastern Ohio Univs. Col. Med., Rootstown, OH 44272, and Nassau County Med. Ctr., East Meadow, NY 11554.

Cholecystokinin (CCK) is a hormone originally found in the gut and more recently located in the central nervous system. Receptors which bind CCK are also present in both of these areas. Behavioral studies have indicated that CCK plays a role in satiety but the relative contribution of neural versus gut CCK is still open to question. A correlation between CCK receptor levels and manipulation of food intake might suggest the importance of a central mechanism. We have compared CCK receptor binding levels in two groups of adult, male rats (eleven animals per group). One group was food deprived for 72 hours, while the other group was fed ad libitum; both groups had water freely available. Body weights of the two groups were matched prior to deprivation, which resulted in a 13% loss of body weight. The animals were sacrificed by decapitation and the brains were rapidly dissected into the following regions: cortex (C), olfactory bulb (O), hippocampus (HI), hypothalamus (HY), midbrain (M), hindbrain (HB) and caudate-putamen (CP). Receptor binding was determined by incubation of crude membrane preparations with 125-I imidoester CCK-8 as the radioligand. Data were expressed as % bound per mg protein. Receptor binding levels fell into three categories in both groups of rats. High levels (>50) were seen in samples of C, CP and O. Low levels (<20) were found in samples of HY, M and HB, whereas an intermediate level (approximately 25) was observed in HI. When the ratio of values for deprived to ad libitum groups was calculated, the greatest effect was in the sample of HY where deprived animals had twice as much CCK receptor binding as the non-deprived animals; an increased level of CCK receptor binding was also seen in HB of deprived rats (ratio: 1.5). In the olfactory bulb, however, a decreased level of CCK receptor binding was observed in deprived rats (ratio: 0.71). Smaller differences were present in the four other regions sampled. Scatchard plots of samples of HY and O demonstrated separate but parallel lines for the two groups of animals, whereas plots of C tissue fell on a single line for both groups. These results demonstrate that food deprivation can change levels of CCK receptor binding in the central nervous system and that the region of greatest change is the hypothalamus which has been shown, in numerous studies, to play a role in food intake and body weight regulation.

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280.3 SUBUNIT STRUCTURE OF INSULIN RECEPTORS IN RAT BRAIN. Kim A. Heidenreich, M. Beth Goens* and Nancy R. Zahniser. Depts. of Medicine and Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Specific binding sites for ^{125}I-insulin have been previously identified in mammalian brain using in vitro radioligand binding techniques. The pharmacological characteristics of ^{125}I-insulin binding to brain membranes are similar to those of more classical target tissues such as liver and adipocytes. In the present study, the regional distribution of specific ^{125}I-insulin binding sites in synaptic and nonsynaptic fractions from various brain regions (olfactory tubercle, cerebral cortex, hippocampus, striatum, hypothalamus and cerebellum) was determined. The synaptic fraction, consisting of primarily synaptic and mitochondrial membranes, and the nonsynaptic fraction, made up of nuclear and capillary membranes, were isolated by differential centrifugation. Specific ^{125}I-insulin binding was relatively uniform throughout the brain regions examined (124 fmol/mg protein). In addition, the binding was evenly distributed between synaptic and nonsynaptic membranes in all the regions except for the olfactory tubercle where there was a 2-fold enrichment of binding (256 fmol/mg) to the synaptic fraction. The affinity of the binding sites (1-10 nM) was similar in all brain regions.

To determine whether the insulin binding sites in rat brain were structurally similar to insulin receptors found in peripheral tissues, membranes from several brain regions were photoaffinity labeled using the photoreactive analog of insulin B2(2-nitro,4-azidophenylacetyl)-des-Phe^{B1}-insulin (NAPA-DP-insulin). This compound was a gift from D. Brandenburg. NAPA-DP-insulin is an agonist with slightly reduced binding affinity and has been used to covalently label insulin receptors in adipocytes and liver. The binding of NAPA-DP-insulin (40 ng/ml) to brain membranes at 16°, followed by photolysis, resulted in the covalent labeling of several proteins determined by SDS electrophoresis and autoradiography. In non-reduced samples, one major protein having an apparent M.W. of 400,000 daltons was specifically labeled with NAPA-DP-insulin. The migration of this protein was not different from radiolabeled insulin receptors on rat adipocytes. When brain samples were exposed to dithiothreitol before electrophoresis, one band having an apparent M.W. of 115,000 daltons was specifically labeled. This pattern of labeling differed markedly from that in adipocytes where labeling occurred primarily in a 125,000 dalton protein and to a lesser extent in 115,000 and 90,000 dalton proteins. The non-reduced (400,000 daltons) and the reduced (115,000 daltons) forms of the receptor were the same in synaptic and nonsynaptic fractions and in all the brain regions examined. Thus, although the brain has high affinity binding sites for insulin, the subunit structure of these binding sites in the brain is clearly different from that of insulin receptors in the periphery.

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280.4 EFFECT OF SULFHYDRYL REAGENTS ON ANGIOTENSIN II RECEPTORS. Fon-Chiu Mia Chen*, and Morton P. Printz* (SPON:A.Vitto). Dept. of Medicine, Univ. of Calif., San Diego, La Jolla, CA 92093

The properties of angiotensin II receptors (ANG II R) may be revealed by the effect of sulfhydryl reagents on ANG II binding. In the present study, we examined the effects of dithiothreitol (DTT), p-hydroxy mercuribenzoate (PHMB), N-ethylmaleimide (NEM), and phenylmercuric acetate (PMA) on ANG II binding. We found that DTT enhanced ANG II binding in the rat brain by increasing the affinity from Kd of 0.4 nM to 0.2 nM without affecting the receptor density. Further studies revealed that DTT not only increased binding level but also facilitated the dissociation of ANG II binding. In the presence of DTT, [125-I]-ANG binding reached equilibrium at about 60 min. The dissociation of bound [125-I]-ANG II from rat brain homogenate was assessed by adding 1 µM unlabeled ANG II to the assay medium; the dissociation half-life was 35 min and rate constant was 0.018 min^{-1}. In the absence of DTT, subsequent dissociation after the addition of unlabeled ANG II displaced only 50% of the specific binding in 60 min; no further dissociation was observed. Although PHMB and NEM did not inhibit ANG II binding, even at concentrations as high as 1 mM, PMA decreased ANG II binding dramatically with IC 50 at 100 µM. In addition, PMA also decreased the dissociation of bound ANG II. No appreciable amount of dissociation was observed when PMA was present. In contrast to the effect on rat brain, DTT decreased ANG II binding in both the pineal and the anterior and posterior pituitary. We also found that ANG II R in the pituitary have a lower affinity than those in the brain regions. This discrepancy may be due to the tissue specificity of ANG II R.

In conclusion, we found that DTT enhances ANG II binding in the rat brain by increasing the affinity, not receptor density. DTT also facilitated the dissociation of bound ANG II from these binding sites. Although both PHMB and NEM did not inhibit ANG II binding, PMA decreased both ANG II binding and its dissociation in the rat brain. In contrast to the effect in rat brain, DTT decreased ANG II binding in the pineal and the pituitary. The effect of the sulfhydryl reagents PMA and DTT may reveal an important aspect of the interaction between ANG II and ANG II receptor.

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280.5 COMPARISON OF CAPSAICIN AND SUBSTANCE P-INDUCED CYCLIC AMP ACCUMULATION IN ISOLATED GUINEA PIG SPINAL CORD. W. Northam*, K. Folkers* and D. Jones. Depts. Anesth. and Pharmacol., The Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284; Institute Biomed. Res., Univ. Texas, Austin, TX 78712 (SPON: J. Story).

Capsaicin (CAP), the principal active ingredient of the hot pepper (genus Capsicum), has been demonstrated to evoke the Ca^{++} dependent release of substance P in tissue slices from the dorsal area of spinal cord (Gamse et al., Life Sci., 25:629, 1979). Since both agents have previously been demonstrated to stimulate adenylate cyclase in rat cerebral cortex, it was the purpose of the present study to examine the effects of CAP and substance P on guinea pig regional spinal cord cyclic AMP accumulation.

Male Hartley guinea pigs (300-500 G) were sacrificed and spinal cords rapidly removed and cut transversely into dorsal and ventral sections. The spinal cord sections were then sliced (300 μm) using a McIlwain tissue chopper and subsequently preincubated for 60 min in oxygenated Krebs-Ringer bicarbonate buffer. Following this period, the slices were placed into incubation flasks for 15 min at which time various agents were added. At the end of the drug incubation period, tissue protein was acid denatured and cyclic AMP measured by radioimmunoassay. All values are picomoles cyclic AMP/mg protein.

CAP (10^{-6} M) significantly increased cyclic AMP accumulation within 2 min following addition, with the maximal increase occurring at 5 min. This increase was sustained for at least 30 min. CAP-induced cyclic AMP accumulation was concentration dependent (EC_{50} 5 x 10^{-8} M) and occurred only in tissue-slices from dorsal spinal cord. In dorsal spinal cord tissue slices, the maximal increase (11 ± 1 to 72 ± 8) occurred at a CAP concentration of 10^{-6} M. CAP did not stimulate cyclic AMP accumulation under Ca^{++} free conditions. Structure activity studies indicated that both the alkyl and substituted aromatic portions of the capsaicin molecule are necessary for this stimulatory effect in dorsal cord.

Substance P (10^{-9} - 10^{-4} M) produced a slight increase in cyclic AMP accumulation in dorsal spinal cord only at higher concentrations. The response was not concentration-dependent. The substance P antagonist (D-Pro^2, D-$Trp^{7,9}$)-substance P did not block the CAP-induced increase in cyclic AMP in dorsal cord. These data indicate that the stimulatory effects of CAP are not mediated by substance P release and are apparently due to the direct effects of CAP on the dorsal spinal cord cyclic AMP system.

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280.6 PEPTIDE RECEPTORS IN GUINEA PIG HEART POSSIBLY INVOLVED WITH AUTONOMIC NERVOUS ACTIVITY. D. C. Manning and S. H. Snyder. Johns Hopkins University Sch. of Med., Dept. of Neuroscience, Pharmacology and Psychiatry, Baltimore, MD 21205.

Bradykinin, a nonapeptide, is one of the most potent algesic substances known and is often used for experimental production of cardiovascular and cutaneous pain states. Bradykinin stimulates both vagal and sympathetic sensory fibres with receptive fields in the heart and is proposed to function in mediating the pain associated with myocardial ischemia. A role for bradykinin in the heart is suggested by the presence of kinin forming and degrading enzymes in myocardial tissue. During myocardial ischemia bradykinin is released from the heart and bradykinin stimulation of cardiac sympathetic afferents and can mimic the excitatory reflexes observed during ischemia.

To determine if bradykinin receptors exist in the heart we have employed ^{3}H-bradykinin in both tissue homogenate receptor binding and autoradiographic techniques. ^{3}H-Bradykinin (52 Ci/mmole) binding in the guinea pig heart exhibits saturable binding to membrane homogenates with a K_D of approximately 15 pM.

Intramural adrenergic neurons of the guinea pig heart are thought to have not only bradykinin receptors but also angiotensin II receptors. Accordingly, we are investigating ^{125}I angiotensin II binding in cardiac tissue homogenates and in heart tissue sections by autoradiography.

280.7 LOCALIZATION OF ^{3}H-(3MeHis^2)THYROTROPIN RELEASING HORMONE RECEPTORS IN THE SEPTAL REGION OF THE RAT BRAIN. S. M. Simasko* and A. Horita* (SPON: J.D. Loeser). Dept. of Pharmacology and Dept. of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195.

Localization of ^{3}H-(3MeHis^2)TRH receptors in the septal area of the rat brain was investigated by producing selective lesions of specific neuronal populations and then measuring the subsequent changes in receptor binding. The septal region is the brain area that has been shown to be the most sensitive brain region in which microinjected TRH antagonizes pentobarbital narcosis (Kalivas, P. W. and Horita, A., J. Pharmacol. exp. Ther. 212:203, 1980). We have sought to identify the precise neuronal elements responsible for this observation. The following lesions were produced to eliminate particular neuronal elements in the septal region: kainic acid (2 μg/2 μl/4 min)injected via a 30 ga. stainless steel tube placed stereotaxically in the septal region (KA-SEP) or the lateral ventricle (KA-LV); and radiofrequency electrolytic lesions (10 mA/40 sec) via a Teflon coated wire (.008 inch diameter) with 1 mm of the tip exposed stereotaxically placed in the medial forebrain bundle (E-MFB) or the fimbria (E-FIM). Receptor number was measured by incubating septal tissue homogenates with 8 nM of ^{3}H-(3MeHis^2)TRH (total binding) and subtracting the binding in the presences of 10 μM TRH (nonspecific binding). The results (expressed as a percent of control) are as follows: control, 100%; KA-SEP, 68%; KA-LV, 87%; E-MFB, 89%; E-FIM, 90%. The decrease after KA-SEP indicates that at least 32% of the receptors are located on cell bodies. The slight drop after KA-LV would indicate that the decrease after KA-SEP is not due to kainic acid leaking into the ventricular system and destroying cells elsewhere in the brain. The failure of the electrolytic lesions to cause alterations in receptor number indicates that the receptors are not located on presynaptic terminals of axons which project through the medial forebrain bundle or the fimbria. Thus it is tentatively concluded that TRH in the septal region acts directly on cell bodies and not through alterations in the release of other neurotransmitters.

280.8 CHARACTERIZATION OF A RECEPTOR FOR TYR-PRO-LEU-GLY-NH_2 (TYR-MIF-1) IN RAT BRAIN, J.E. Zadina*, A.J. Kastin, E. Krieg*, and D.H. Coy*. Neuroendocrine Lab., VA Medical Center and Tulane University School of Medicine, New Orleans, LA 70146

Pro-Leu-Gly-NH_2 (MIF-1), the C-terminal tripeptide of oxytocin, has been shown to be active in numerous behavioral tests and clinical situations. Attempts to develop a radioimmunoassay (RIA) for MIF-1 produced an antibody that cross-reacted much better with Tyr-MIF-1 than with MIF-1. In the RIA using this antibody, material similar to Tyr-MIF-1 was found in several parts of the brain and levels of immunoreactivity changed after pinealectomy and at different times of day (Brain Res. Bull. 7, 697, 1981).

We now demonstrate a binding site in rat brain that is saturable and specific for Tyr-Pro-Leu-Gly. Rat brains were removed, rapidly dissected on ice, and homogenized in sucrose. Crude P2 synaptosomal fractions were prepared by removing the 1000 g pellet and centrifuging the supernatant at 30,000 g. These membranes were incubated in 25 mM sodium phosphate buffer with the enzyme inhibitor Bestatin and ^{125}I-Tyr-MIF-1 in the presence or absence of unlabeled Tyr-MIF-1. Binding in the presence of 10^{-5}M unlabeled Tyr-MIF-1 was defined as nonspecific binding. Other peptides and amino acid residues were tested and failed to effectively compete for ^{125}I-Tyr-MIF-1 binding at this concentration. Substances tested included oxytocin, MIF-1, two analogs of MIF-1 (pGlu-Leu-Gly and cyclo Leu-Gly), isotocin, lysine vasopressin, TRH, somatostatin, LHRH, Met- and Leu-enkephalin, Tyr-Pro, Pro-Leu and Tyr. Binding reached equilibrium at 30-40 min at 23°C and at about 4 hr on ice, after which it was relatively stable for at least 18 hr. Binding was linear with protein from 200 μg to at least 1.2 mg protein per tube. Bound and free ^{125}I-Tyr-MIF-1 were separated by rapid filtration on glass fiber filters. Scatchard analysis of the striatum-thalamus area in a 4°C incubation revealed the presence of binding sites for Tyr-MIF-1 with an apparent Kd less than 100 nM and maximum number of sites in the range of 40 pmol/g tissue. Comparison of binding data generated by varying the labeled peptide to that produced by varying the unlabeled peptide indicated that iodination did not appreciably affect the affinity of the ligand for the receptor. Analysis of several brain areas revealed a differential distribution of the binding sites, ranging from relatively high concentrations in striatum and cortex to low concentrations in pons-medulla. Together with the previously published RIA data, the discovery of a receptor for Tyr-Pro-Leu-Gly-NH_2 supports the concept of the presence of a novel peptide and its receptor in the brain.

280.9 LACK OF DOWN REGULATION OF INSULIN RECEPTORS BY INSULIN IN NEURON-ENRICHED PRIMARY CULTURES OF RAT BRAIN. F.T. Boyd, Jr.*, R. Deo*, M.K. Raizada*. (SPON: W. Dawson). Dept. of Physiology, Univ. of Florida, Gainesville, Florida 32610.

In previous studies we have demonstrated the presence of insulin-specific receptors in primary cultures of rat brain. Binding of insulin to these receptors is associated with insulin's stimulation of nucleotide incorporation into macromolecules. It is possible that the insulin effector system in the brain may be substantially different from the insulin system in the periphery because of the presence of blood-brain barrier and the unique metabolism of the CNS. The present study was undertaken to investigate possible differences between CNS and peripheral insulin receptor regulation.

Brains from one day old rats were trypsinized and dissociated cells were plated in polylysine coated culture dishes in Dulbecco's Modified Eagles Medium containing 10% fetal bovine serum. Inhibition of cell division and subsequent disintegration of multiplying cells, predominantly of non-neuronal origin, was achieved by treatment of cultures with cytosine arabinoside for 2 days. These cultures contained up to 80% neurons which developed extensive networks of neurites.

Incubation of 11 day old neuron-enriched brain cell cultures for 24 hours with varying concentrations of insulin (0.167-8.3μM) failed to cause a decrease in the specific binding of ^{125}I-insulin. However, in confluent cultures of mouse skin fibroblasts similar concentrations of insulin produced a dose dependent decrease of ^{125}I-insulin binding. Incubation of neuron-enriched cultures with 8.3μM insulin for varying durations up to 24 hours did not show an effect on the binding. In contrast, a time-dependent decrease in the binding of insulin was observed in cultures of skin fibroblasts. Addition of 0.56μM Tunicamycin, an inhibitor of protein glycosylation, which caused a time dependent decrease in the ^{125}I-insulin binding in fibroblasts, failed to reduce insulin binding in neuron-enriched brain cell cultures.

These observations indicate that i) brain insulin receptors are not down-regulated by insulin unlike peripheral insulin receptors and ii) glycosylation of insulin receptors may not be an important step in the regulation of their surface expression in the brain. (Supported by a grant from American Diabetes Association.)

280.10 HIGH AFFINITY BINDING OF SECRETIN TO RAT BRAIN MEMBRANES. R.T. Fremeau*, R.T. Jensen*, T.L. O'Donohue* and T.W. Moody. Dept. Biochemistry, The George Washington University School of Medicine, Washington, D.C. 20037; Sec. Gastroenterology, NIAMDD and Lab. Clin. Sci., NIMH, Bethesda, MD 20205.

Secretin, a 27 amino acid peptide, is biologically active in the central nervous system (CNS) and periphery. In the periphery it causes the release of pancreatic enzymes, in particular, amylase (Mutt, V. et al., Eur. J. Biochem., 15: 513, 1970), whereas in the brain, secretin reduces open field activity (Charlton, C.G. et al., Society for Neuroscience, 7: Abstract #163.7,1981). Also endogenous secretin like peptides have been detected in the periphery and CNS (O'Donohue, T.L. et al., Proc. Natl. Acad. Sci. USA, 78: 5221, 1981). Because secretin-like peptides may function as important regulatory agents in the CNS, in addition to the periphery, we undertook the characterization of secretin receptors in mammalian brain.

Secretin was radiolabeled and ^{125}I-secretin purified using gel filtration, cation exchange chromatography and high pressure liquid chromatography techniques. The radiolabeled peptide possessed appreciable biological activity. ^{125}I-secretin bound with high affinity (Kd=0.4 nM) to rat brain homogenate. This high affinity binding was noncooperative, saturable and reversible (≈5 fmol sites/mg protein). Regional distribution studies indicated that the density of sites was 20-fold greater in high regions such as the cerebellum than low regions such as the medulla-pons. Intermediate densities were present in the cortex, hippocampus, striatum, thalamus and hypothalamus. Pharmacological studies indicated that ^{125}I-secretin was inhibited in a dose dependent manner by unlabeled secretin strongly (IC_{50}=0.5 nM) and weakly by VIP (IC_{50}=0.3 μM) and GIP (IC_{50}=5 μM). Other peptides and neurotransmitters tested did not compete for the ^{125}I-secretin binding sites. These data indicate that rat brain membranes may contain unique receptors which mediate the effects of secretin-like peptides.

280.11 CHARACTERIZATION OF SOMATOSTATIN RECEPTORS IN RAT BRAIN USING THE NONREDUCIBLE SOMATOSTATIN ANALOG CGP 23996. Andrew J. Czernik* and Barbara Petrack* (SPON: R. A. Lovell). Dept. of Biochemistry, New York Medical College, Valhalla, N. Y. 10595 and Research and Development Department, Pharmaceuticals Division, CIBA-GEIGY Corp., Ardsley, New York 10502.

Specific, saturable, high affinity binding sites for somatostatin (SS) have been identified in P_2 synaptosomal preparations of rat brain using the nonreducible SS analog, des-Ala^1, Gly^2-desamino-Cys^3-[Tyr^{11}]-dicarba^{3,14}-SS (CGP 23996). This analog labeled with ^{125}I, was significantly more resistant to degradation than ^{125}I-N-Tyrosinyl-SS and [^{125}I-Tyr^{11}]SS during binding assays performed at 37 °C. The extent of radioligand degradation as measured by both antibody binding and by TLC, was 40, 70 and 95% for ^{125}I-labeled CGP 23996, [Tyr^{11}]SS and N-Tyrosinyl-SS, respectively, in the absence of proteolytic enzyme inhibitors. Bacitracin (20 μg/ ml) afforded further protection from degradation of the radioligands, but trasylol (250 U/ml) and phenylmethylsulfonyl fluoride (1 μg/ ml) were ineffective. The degradation of ^{125}I-CGP 23996 and [^{125}I-Tyr^{11}]SS, but not ^{125}I-N-Tyrosinyl-SS, was also reduced by 5 mM $MgCl_2$. In the presence of bacitracin and $MgCl_2$, ^{125}I-CGP 23996 was almost fully protected (3% degradation), permitting characterization of SS receptor binding at physiologic temperature. Specific binding of ^{125}I-CGP 23996 reached equilibrium in 30 min and was stable for an additional 30 min. Scatchard analysis of binding data was linear, yielding a K_D = 2.4 ± 0.1 nM and a Bmax = 450 ± 30 fmol/mg protein. SS exhibited competitive inhibition of ^{125}I-CGP 23996 binding. The regional distribution of binding sites in rat brain was variable, with the highest amounts in cerebral cortex and hippocampus and little binding in cerebellum and pons/medulla. There was a good correlation between relative potency values of SS analogs determined in the binding experiments with those reported in bioassay tests, while other neuropeptides were ineffective in displacing specific binding. Representative IC_{50} values in our binding assay were as follows: [5-F-D-Trp^8]SS = 0.9 nM; SS = 1.5 nM; CGP 23996 = 2.0 nM; [Leu^8, D-Trp^{22},Tyr^{25}]SS-28 = 4.9 nM. Divalent cations (5 mM) influenced SS receptor binding. Mg^{2+} increased specific binding of ^{125}I-CGP 23996 while Ca^{2+} and Mn^{2+} were inhibitory. Divalent cations also influenced the affinity of "mini-SS" analogs in our binding assay. The relative potencies of [Aha^5,D-Trp^8]-cyclo[5-11]SS (IC_{50} = 21 nM) and des-AA^{1,2,4,5,12,13}[D-Trp^8,D-Cys]SS (IC_{50} = 77 nM) were increased 10- and 30-fold, respectively, in the presence of 5 mM $MgCl_2$ or $CaCl_2$. Our results demonstrate the presence of SS receptors in rat brain and support the proposal that SS is a modulator of neurotransmission.

280.12 EFFECT OF 2.5% SODIUM CHLORIDE INTAKE ON RAT BRAIN AND ADRENAL ANGIOTENSIN II RECEPTORS. R.C. Speth, C.M. Ferrario, R.R. Smeby*, R. Singh*, J. Krontiris-Litowitz* and A. Husain*. Research Div., Cleveland Clinic Fdn., Cleveland, OH 44106

The peripheral renin-angiotensin system (RAS) plays an integral role in fluid and electrolyte balance and undergoes functional changes in response to alterations in body fluid electrolytes. Increased intake of NaCl decreases peripheral RAS activity and adrenal angiotensin II (ANG II) receptor density and increases vascular ANG II receptor density; however,the effects of this stimulus on brain ANG II receptors has not been established. Male Sprague-Dawley rats(150-200 g) housed individually were fed Purina rat chow. The control group (CT,n=9) was given tap water ad lib, while the experimental group (Hi NaCl,n=8) was given a 2.5% sodium chloride solution instead of tap water. After a 13 day treatment period, Hi NaCl rats showed signs of severe dehydration, including a 5% increase in hematocrit and a 23% loss of body weight. Moreover, plasma sodium levels were drastically elevated in Hi NaCl rats (P<0.001). Plasma renin activity measured from blood collected after decapitation, averaged 19.9±6.3 ng ANG I/ml/hr in Hi NaCl rats and 27.4±15.4 ng ANG I/ml/hr in CT rats.

Specific (1 μM ANG II displaceable) ^{125}I-ANG II binding to brain and adrenal ANG II receptors was determined at 5 concentrations of ^{125}I-ANG II ranging from 0.1 to 1.0 nM. Consistent with previous reports of other investigators, there was a marked reduction in ^{125}I-ANG II binding sites in the adrenal of the Hi NaCl rats, 214±47 vs 425±129 fm/mg protein(P<0.001) in CT rats, with no change in binding affinity. In contrast, the brain (hypothalamus- thalamus-septum-midbrain region) of Hi NaCl rats showed an increased ^{125}I-ANG II binding site density (10.7±1.4 vs 9.4±0.7 fm/mg protein), which while small (14%) was significant (P<0.05). Brain ^{125}I-ANG II binding affinity did not differ between the two groups. These results indicate that severe sodium chloride loading in rats causes relatively small alterations in brain ANG II receptors despite the fact that this treatment causes profound alterations in adrenal ANG II receptors.It is also possible though that changes in discrete brain nuclei in response to sodium chloride loading were masked by the large amount of tissue used. Supported by USPHS Grant (HL 27568).

280.13 SUBSTANCE-P RECEPTOR ON RAT SUBMAXILLARY GLAND. S.W. Bahouth*, J. M. Stewart[1] and J. M. Musacchio (SPON: M.M. Puig). Dept. of Pharmacology, New York Univ. Med. Ctr., New York, NY 10016 and [1]Dept. of Biochemistry, Univ. of Colorado School of Medicine, Denver, CO 80262.

Substance-P (SP) is a putative neurotransmitter that has numerous central and peripheral pharmacological effects. Attempts to label the SP receptor with [^{3}H]SP have been hampered by the instability of the ligand that is readily oxidized to [Met-sulfoxide^{11}]SP upon exposure of a dilute solution to air. Here we report the use of a more stable SP analog [Tyrosine^1, Norleucine^{11}]SP which was synthetized by solid phase synthesis by published methods. This peptide acts on the guinea pig ileum receptor with an ED_{50} of 5 nM and shows cross-desensitization with SP and its analogs.

The peptide was iodinated by a modification of the chloramine-T method and the mono iodo derivative was purified by HPLC on a Waters reverse phase C_{18} column. [I-Tyr^1,Nleu^{11}] SP has an ED_{50} of 10 nM on the guinea pig ileum and it also shows cross-densensitization with SP and analogs.

Rat submaxillary glands were homogenized in 20 mM HEPES (N-2-Hydroxyethylpiperazine-N-2-ethanesulfonic acid) pH=7.4 and centrifuged at 37,000 g. The pellet was resuspended in 20 mM HEPES, 0.1 mM TLCK (N-p-tosyl-1-lysine chloromethyl ketone) and TPCK (L-1-tosylamide-2-phenylethyl chloromethyl ketone), incubated at 20°C for 15 min to inhibit trypsin and chymotrypsin like enzymes and then centrifuged at 37,000 g. The resulting pellet was resuspended in the binding medium containing 95 mM NaCl, 3.75 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 0.05 mg/ml chymostatin and 0.3 mg/ml bacitracin. The binding was performed at 20°C for 20 min and terminated by filtering on Whatmann GF/B filters.

Scatchard analysis of [^{125}I]Tyr^1,Nleu^{11}-SP binding to submaxillary gland homogenate shows a K_D of 1.3 nM and a single binding site with a B_{max} of 58 fmole receptor per mg protein. SP and its analogs compete with the label and their IC_{50} have the same order of potency seen when peptides compete for [^{3}H]SP binding to salivary gland homogenate. This work was supported in part by PHS grants DA02013, MH29591 and MH 17785 to J.M.M. and NS 09199 to J.M.S.

281.1 SIMILAR HEAD TURNING RESPONSE INDUCED BY UNILATERAL INTRA-CAUDATE TRH AND DOPAMINE INJECTION IN THE CAT. F. Malouin*, P. Deshaies* and P. J. Bédard. Lab. recherche en Neurobiologie, Université Laval and Hôpital de l'Enfant-Jésus, 1401, 18e Rue, Québec, Qué. CANADA, G1J 1Z4.

Unilateral injection of TRH 10 ug in 5 ul in the head of the caudate nucleus in cats induced a head turning response similar in direction and intensity to the effect of 10 ug of dopamine (DA) injected in the same site. Injection of either substance in the central part of the head of the caudate induced contralateral turning whereas more laterally placed injections produced ipsilateral responses. The DA-induced head turning was completely suppressed by haloperidol, 3 mg/kg i.m. The response to TRH however was potentiated by haloperidol but unaffected by cyproheptadine. These results suggest that although TRH has dopamine-like effects, this action is not mediated through dopamine or serotonin receptors. Neither DA or TRH injected directly into the caudate nucleus modified the number of head movements. Haloperidol but not cyproheptadine pre-treatment, decreased the head motility index providing evidence that postural symmetry is modulated in the caudate nucleus whereas motility appears to involve dopaminergic mechanisms located elsewhere.

281.2 PITUITARY RESPONSIVENESS TO TRH IN LACTATING LONG-EVANS RATS. D. W. McKay* and K. Brown-Grant* (SPON: R. Neuman). Fac. of Med., Memorial Univ. of Nfld., St. John's, Nfld., A1B 3V6.

Suckling is known to induce increases in plasma concentrations of prolactin (PRL) and thyrotropin (TSH). Recently, it has been reported that 10 minutes of suckling exposure following a short period of pup deprivation enables the pituitary to release substantial amounts of PRL in response to doses as little as 2 ng of thyrotropin releasing hormone (TRH), (Grosvenor and Mena, Endocrinology 107: 863, 1980). Studies were, therefore, undertaken to examine the TSH response to TRH in the suckled lactating rat. All litters of lactating rats were equalized to 10 pups. In the initial experiment, the mothers were atrially catheterized under ether anesthesia. Experiments were performed 2 days after catheterization. On the day of treatment, all mothers were pup deprived for 6 hours after which time an initial blood sample was collected. Mothers then were either: 1) allowed 10 minutes of pup contact, or 2) undisturbed for 10 minutes until the next blood sample was taken. Following a blood sample at 40 minutes, all lactaters were injected via catheter with 100 ng TRH (Hoechst). Blood samples were collected at 10 minute intervals for an additional 50 minutes. TSH was measured in plasma by radioimmunoassay with the use of materials supplied by NIAMDD. In a second study, non-catheterized rats were also pup deprived for 6 hours following which the mothers either: 1) remained undisturbed, or 2) were allowed 10 minutes of pup contact. After an additional 10 minutes, each lactater received an injection of 1 ug TRH i.p. All rats were decapitated 20 minutes after injection and trunk blood was collected. In this study, milk content of each pup's stomach was graded on a simple scale of 0-3 to determine the occurrence of milk let down.

In the catheterized animals suckling was not associated with any alteration of the thyrotroph to TRH stimulation, as the administered dose resulted in similar increases of plasma TSH (*2344 ng/ml vs. 2032 ng/ml*). However, in the non-catheterized lactater, suckling produced a more variable TSH response to 1 ug of TRH. In those rats which demonstrated milk let down (5/7), TSH levels remained low (265 ng/ml) while in those suckled females whose pups had low stomach milk content (2/7) and in non-suckled controls (5/5), the TSH response to TRH was marked (4139 ng/ml and 1940 ng/ml, respectively). Since results of other studies have shown that 1 ug of TRH greatly enhances the release of TSH, the results of the present study were unexpected. These results suggest that the sensitivity of the thyrotroph to TRH stimulation may be altered under those conditions when suckling is of sufficient intensity to induce milk let down. (Supported by MRC grant to K. B-G.)

281.3 ALTERATIONS OF CNS THYROTROPIN-RELEASING HORMONE (TRH) FOLLOWING CONVULSIVE (ECS) AND SUBCONVULSIVE (SCS) ELECTROSHOCK IN THE RAT. M.J. Kubek, J.L. Meyerhoff, and A. Sattin Dept. of Anatomy and Inst. Psychiatric Res. Indiana Univ. Sch. of Med., and V.A. Hosp., Indianapolis, IN 46233, and Dept. Med. Neurosciences, Walter Reed Army Inst. of Res. Wash. D.C. 20012.

TRH has been shown to exert several neurophysiological and neuropharmacological effects in the CNS independent of its neuroendocrine function. Among these is a claimed antidepressant effect. Recently, we have shown (Neurosci. Abstr. 7:379, 1981) that ECS, given in a temporal paradigm used clinically, can induce marked and sustained elevations of TRH in specific CNS loci in the rat. In order to more clearly define the specificity of the TRH response to ECS we examined the effect of SCS on TRH content in several CNS areas.

Three groups of 6-7 male S-D rats (150-180 g) were given ECS (75 mA constant current), SCS (5 mA constant current) or sham ECS (without current) for 0.4 sec at 4 PM every other day for a total of 5 treatments. ECS produced tonic-clonic seizures. SCS produced a startle response, vocalization, and brief motor excitation. All rats were decapitated 48 ± 3 hrs after the last treatment. Brains were removed, immediately dissected, weighed, and frozen on dry ice. TRH was assayed by specific RIA following HAc extraction and results expressed as pg/mg tissue (mean±SEM). Data were analysed by ANOVA for repeated measures on log-transformed data followed by comparisons among and between regions.

ECS markedly increased TRH content over sham in amygdalapiriform (Ayp)(11.37±1.44 vs 22.14±1.84, $p<0.01$), hippocampus (HC) (6.15±0.52 vs 15.50±1.80, $p<0.01$), cortex (Ctx) (0.83±0.07 vs 1.48±0.13, $p<0.01$), and slightly in striatum (ST) (5.85±0.82 vs 7.84±0.71, $p<0.05$). Of particular interest was the observation that SCS had no significant effect on regional TRH except in ST wherein a 69% ($p<0.01$) elevation was observed 48 hrs following SCS. When compared to SCS, ECS induced significant increases in TRH content in Ayp ($p<0.01$), Ctx ($p<0.05$), and HC ($p<0.01$) but not in ST. In fact, the ECS response in ST was somewhat lower (7.84±0.71 vs 9.87±1.26). Neither ECS nor SCS had any effect on TRH in the n. accumbens (Acc).

These results: (1) demonstrate for the first time an increase in cerebral cortical TRH following ECS, (2) shows that SCS has no effect on TRH in Ayp, HC, or Ctx at a time when TRH in these areas is elevated following ECS, (3) indicate that TRH in Acc is not involved in the long-term effect of either ECS or SCS, (4) suggest that TRH in ST is altered by SCS or its associated behavioral effects, and (5) confirm and extend our previous findings that alternate-day ECS induces elevations of TRH in specific CNS loci. Supported by Grant AM-28260.

281.4 FURTHER STUDIES ON THE IN VITRO PROGESTERONE-STIMULATED LHRH RELEASE FROM IMMATURE RAT HYPOTHALAMI SUPERFUSED IN VITRO K. Kim and V.D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Previous work in this laboratory has demonstrated that the intermittent infusion of progesterone (P) is highly effective in stimulating LHRH release in vitro from superfused hypothalamic fragments from immature ovariectomized, estradiol-primed (OVX+E_2) rats (Kim and Ramirez, Endocrinology, 1982, in press). The present study was designed to examine: 1) the requirement of E_2-exposure; 2) the tissue site of action (MBH vs POA-AHA); and 3) the effectiveness of 5α-dihydroprogesterone (DHP), a P metabolite, on LHRH release.

Immature 28-day-old female rats were ovariectomized and implanted with silastic capsules containing estradiol. At 30 days of age, animals were decapitated (10:00-10:30 a.m.), and the mediobasal hypothalamic-anterior hypothalamic-preoptic tissue (MBH-AHA-POA) units were removed. P or DHP were dissoved in ethylene glycol and then diluted 10^5 times in superfusion medium to a final concentration of 10 ng/ml. Four tissue fragments/chamber were used, and infused P (or DHP) delivered in an intermittent mode (10-min on, 20-min off). Effluents were collected at 10 min intervals for 210 min, and experimental treatments started after 1-hr control period. LHRH was determined in effluents and post-superfusion tissue extracts by a specific and sensitive radioimmunoassay. Each experiment was replicated four times.

P was effective in stimulating episodic LHRH release from hypothalamic tissues previously exposed to estradiol, confirming once more our previous results. The mean LHRH release rate (pg/mg/min x 10^{-2}) during the intermittent P infusion was significantly greater in OVX+E_2 than in OVX (X±SE, 1.27 ± 0.07 vs 0.66 ± 0.04, $p<.01$). When either MBH or POA-AHA were perifused separately, only MBH fragments responded with increased LHRH release, while POA-AHA failed to do so (% increase over its pre-infusion values, 149 ± 5 vs 101 ± 9, $p<.01$). A similar comparison of pre- vs post-infusion values in the preparations infused with P or DHP demonstrated that P significantly ($p<.01$) increase LHRH release (70 % over its pre-infusion values), whereas DHP failed to show a significant increase (20 % over its pre-infusion values).

It appears then that it is P, and not DHP, the active steroid in stimulating LHRH release from hypothalamic tissues superfused in vitro. Moreover, these results indicate that P requires a background of estradiol pre-treatment to exert its effects, and may act directly on the nerve terminals of the MBH to activate the release phase of the LHRH neurosecretory process.

281.5 CORTICOTROPIN RELEASING FACTOR (CRF) DEPOLARIZES AND EXCITES PYRAMIDAL NEURONS OF THE HIPPOCAMPAL SLICE PREPARATION. J.B. Aldenhoff*, D.L. Gruol and G.R. Siggins. A.V. Davis Center, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

CRF, a 41 residue peptide recently sequenced and synthesized by W.Vale and colleagues (Science 213:1394, 1981), elevates plasma ACTH and β-endorphin levels, and raises systemic blood pressure when injected I.c.v. It has been suggested that the role for this peptide is to prepare the organism for stressful situations. Reasoning that central CRF might also mobilize the CNS for such emergencies, we tested the electrophysiological effect of synthetic CRF (kindly provided by Drs. W. Vale and J. Rivier) on CA1 and CA3 pyramidal cells of the rat hippocampus *in vitro*. Hippocampal slices were prepared for intracellular recording as previously described (Siggins and Schubert, Neurosci. Lett., 23:55, 1981) and completely immersed in a chamber under continuous superfusion with carbogenated artificial CSF solution (33-36°C). Recording micropipettes contained 4M K-acetate. CRF, applied by superfusion (0.2-4 μM), depolarized the eleven CA1 and CA3 neurons studied (in all 22 tests), by 3 to 12 mV, with marked increases in action potential discharge or bursts, but no apparent decrease in input resistance. In 5 cells studied, CRF also reduced the after-hyperpolarizations following spontaneous spike bursts or after current-evoked action potentials, when superfused in concentrations of 0.06-0.5 μM. This effect could be elicited by CRF concentrations which did not alter membrane potential. Since these afterpotentials may arise from calcium-dependent potassium conductances (Hotson and Prince, J. Neurophysiol. 43:409, 1980; Schwartzkroin and Stafstrom, Science 210:1125, 1980), it is possible that CRF either may alter such potassium conductances directly or indirectly through alteration of calcium conductances. Our finding of an excitatory action of CRF on pyramidal neurons is consistent with its proposed role in mobilizing the CNS, via a direct activation of central neurons. In the hypothalamus or pituitary, such an excitatory mechanism could also lead to release of neurohormones such as ACTH and β-endorphin. This work was supported by grants from NIH (AM-26741) and the Alexander-von-Humboldt Stiftung.

281.6 TETRODOTOXIN SUPPRESSES BASAL AND STIMULATED SOMATOSTATIN SECRETION FROM CULTURED BRAIN CELLS. R.A. Peterfreund and W.W. Vale. Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037 and Department of Neurosciences, UC San Diego, La Jolla, CA 92093

We have previously reported that collagenase dispersed brain cells from 18 day fetal rats maintained in long term culture exhibit a measurable basal secretion of somatostatin-like immunoactivity (SSLI). The cholinergic agonist carbachol, the 41 residue ovine peptide corticotropin-releasing factor (CRF) and the phorbol ester derivative PMA were found to significantly augment basal secretion. The present experiments determined the effect of tetrodotoxin (TTX), a fish poison believed to block sodium channels in excitable membranes, on basal and stimulated SSLI release from cultured cells. Experiments were typically performed on the tenth through twelfth day in culture. The serum supplemented culture medium was washed from the dishes and the cells were incubated for one hour at 37° C in Hepes buffered Krebs Ringer solution with or without test substances. TTX (10^{-8} - 10^{-6} M) significantly suppressed basal secretion. After washing, TTX treated cells responded normally to a depolarizing stimulus (59 mM K+). Pretreatment with TTX eliminated secretion stimulated by carbachol (100 μM) and CRF (100 nM) and markedly decreased the effect of PMA (100 nM) administration. Pretreatment with either 1 mM cobalt ion in a medium lacking added calcium or with the GABA agonist muscimol (5 μM) also significantly diminished response to PMA. Together with our previous observation that cobalt ion suppressed basal and carbachol or CRF stimulated secretion, the present findings suggest that cultured somatostatin neurosecretory cells possess an intrinsic level of electrically induced release or are excited to secrete by the influence of other cells in the heterogenous cell population. Cholinergic agonists or CRF apparently act at a site proximal to the axon terminus of the somatostatin cell to augment SSLI secretion via a mechanism mediated by sodium and calcium influx. Since PMA responses are diminished by TTX, cobalt and muscimol, the data are also consistent with a mechanism of action for PMA mediated at least in part by sodium and calcium channels and which can be affected by monoamine neurotransmitter systems. These observations support the hypothesis that PMA and ovine CRF mimic the effects of endogenous modulator substances in rat central nervous tissue.

281.7 SOMATOSTATIN AND ANALOGS INHIBIT ENDOGENOUS SYNAPTIC PLASMA MEMBRANE PROTEIN PHOSPHORYLATION *IN VITRO*. L.A. Dokas, H. Zwiers*, D.H. Coy* and W.H. Gispen*. Departments of Neurosciences and Biochemistry, Medical College of Ohio, Toledo OH 43699; Department of Medicine, Tulane University, New Orleans LA 70112; Institute of Molecular Biology and Rudolf Magnus Institute for Pharmacology, State University of Utrecht, The Netherlands.

Given the evidence that the hippocampus is a functional site of action for somatostatin, we have examined whether somatostatin may affect one neurochemical process, synaptic plasma membrane (SPM) protein phosphorylation, believed to modulate synaptic efficacy. Addition of somatostatin to hippocampal SPM preparations *in vitro* decreases subsequent phosphorylation of specific protein bands. 10^{-4}M somatostatin inhibits the phosphorylation of protein bands with apparent molecular weights between 10,000 and 20,000 daltons and, to a lesser extent, 48,000 daltons (B-50) and 52,000. Increasingly greater degrees of inhibition are seen in response to somatostatin-28 and [D-Trp^8]-somatostatin. Inhibition of B-50 protein phosphorylation in the presence of [D-Trp^8]-somatostatin is most prominent in SPM preparations from the hippocampus and amygdala, with lesser degrees of inhibition seen in the cortex and hypothalamus. Addition of [D-Trp^8]-somatostatin to an ammonium sulfate-precipitated fraction (ASP 55-80) from cortex only slightly inhibits endogenous B-50 phosphorylation, although the ASP55-80 fraction becomes more sensitive to ACTH. Injection of [D-Trp^8]-somatostatin intracerebroventricularly into rats does not induce excessive grooming behavior, but injection of 10 μg of [D-Trp^8]-somatostatin does result in barrel rotation. These results suggest that somatostatin and congeners affect SPM protein phosphorylation in a manner different from that of ACTH, presumably involving membrane sites binding somatostatin.
Supported in part by Grants NS11718 and AM18370 from NIH.

281.8 VASOPRESSIN-INDUCED HYPOTHERMIA: STRUCTURE-ACTIVITY RELATIONSHIPS. G. Meisenberg* and William H. Simmons* (SPON: R. Schmidt). Dept. of Biochemistry, Loyola Univ. Med. School, Maywood, IL 60153.

Vasopressin causes a dose-dependent hypothermia after intracerebroventricular injection in rats. To obtain information concerning the mechanism of this effect, a structure-activity study was performed with the following neurohypophyseal hormones and analogs: Arginine-vasopressin (AVP), arginine-vasotocin (AVT), oxytocin, isotocin, 1-deamino [4-α-aminobutyric acid]-arginine-vasopressin (d ABU) and [des-glycinamide^9]- lysine-vasopressin (DGLVP). Body temperature was determined with a rectal thermistor at an ambient temperature of 23°C 10 minutes after the injection. AVP caused significant hypothermia after doses of 100 ng and 1μg, but not after 10 ng. AVT caused the same effect, but was somewhat less potent. Oxytocin was active at 10 and 20μg, but inactive at 1μg. Isotocin, a naturally occurring neurohypophyseal hormone with predominantly oxytocic properties, was inactive at 20μg. dABU, a synthetic analog of vasopressin with enhanced antidiuretic and reduced pressor activity, was less potent than both vasopressin and vasotocin. DGLVP, which is almost devoid of peripheral hormonal effects but capable of modifying memory processes and the development of drug tolerance, was inactive at doses up to 20μg. These results show that the hypothermia induced by centrally injected vasopressin is mediated by receptors that resemble the vasopressin receptors in peripheral tissues, especially those that mediate the increase in blood pressure in the rat. The mechanisms of this effect are unrelated to those underlying the effects of vasopressin on memory and tolerance formation. (This work was supported by USPHS grants AM30970 and HL28710).

281.9 CHANGES IN IMMUNOREACTIVE VASOPRESSIN CONCENTRATIONS IN THE BRAIN OF THE RAT IN RESPONSE TO ENDOTOXIN. N.W. Kasting and J.B. Martin. Dept. of Neurol., Mass. Gen. Hosp. & Harv. Med. School, Boston, MA 02114.

Arginine vasopressin (AVP) has been hypothesized to have neuromodulator or neurotransmitter roles in various areas of the central nervous system (CNS) on the basis of neuroanatomical and neurophysiological evidence. AVP has been suggested to act as an antipyretic neuromodulator in the septal area during fever. These experiments were designed to measure changes in immunoreactive AVP concentrations in various brain regions of the rat in response to endotoxin. Rats were surgically implanted with an intra-atrial catheter, allowed to recover to preoperative body weight and tethered in an isolation box. Rats received E. Coli (150 mg/kg) iv. Three groups of rats were studied 1) control-no endotoxin 2) 60 min after endotoxin (hypothermic stage) 3) 330 min after endotoxin (hyperthermic stage). Rats were sacrificed and punches were taken from various brain areas, extracted in 2 N acetic acid, lyophilized and resuspended in assay buffer. AVP radioimmunoassay was performed on appropriate dilutions. Protein determinations were also done on the samples. Each punch was about 100 µg protein. Control values in pg AVP/µg protein were as follows: posterior pituitary 714.9; median eminence 50.8; SON 7.5; PVN 5.9; SCN 2.0; medial septum 0.25; lateral septum 0.15; preoptic/anterior hypothalamus 0.24; amygdala 0.016; caudate 0.098; hippocampus 0.015; cerebellum 0.006; brainstem (NTS) 0.017 and plasma 1.49 pg/ml. No detectable charges occurred in the following areas during the hypothermic stage: SON, PVN, SCN, median eminence, medial septum, lateral septum, hippocampus, amygdala, preoptic/anterior hypothalamus, cerebellum or brainstem (NTS). Increases in AVP concentrations occurred in plasma and posterior pituitary whereas a decrease occurred in the caudate nucleus. During the hyperthermic phase no changes compared to control were seen in median eminence, SON, PVN, SCN, medial septum, amygdala, hippocampus, or brainstem (NTS). Increases occurred in plasma, posterior pituitary, preoptic/anterior hypothalamus and cerebellum whereas decreases occurred in lateral septum, and caudate. These data indicate endotoxin stimulates changes in AVP turnover or release in several brain regions. NWK is a Fellow of MRC of Canada. Supported by NIH grant AM 26252.

281.10 REINNERVATION OF ADULT MUSCLE AND NEONATAL DEVELOPMENT OF MOTOR ACTIVITY ENHANCED BY ACTH PEPTIDES. G.R. ACKER*, C. SAINT-COME* AND F.L. STRAND. BIOLOGY DEPT. NEW YORK UNIVERSITY, NEW YORK 10003.

Adrenocorticotropin (ACTH 1-39), 0.2 U daily I.P., improves the mode of recruitment of low threshold motor units during regeneration of crushed peroneal nerve of adult rats. The nerve was stimulated repetitively (1-2 Hz) just above threshold strength. Motor unit size was determined by calculating the average increment in twitch tension amplitude and by calculating the percentage of total tension amplitude represented by each incremental response. Restoration of uniform tension increment was coupled with a beneficial decrease in mean unit size in animals treated with ACTH 1-39, resulting from orderly reorganization of small size motor units. Control animals showed a persistant irregularity in the mode of recruitment together with large unit size. Neonatal rats (1-21 days) were injected with ACTH 4-10 (OI 63) or substituted ACTH 4-9 (Org 2766), 0.1 µg/kg daily I.P. Spontaneous and cold-stressed motor activity were measured using an animal activity monitor. Grasping and releasing responses were measured using a horizontal rod suspended by two poles. The ability to climb up a vertical wire mesh wall was also measured. Neuromuscular contraction responses were determined using an in situ nerve-muscle preparation. Preliminary results reveal that motor activity after cold stress, and grasping and releasing responses were enhanced, and the force of tetanic contractions was increased in ACTH treated animals up to two weeks of age. No differences were found in spontaneous motor activity or climbing ability.

281.11 CARDIOVASCULAR RESPONSES TO INTRAHYPOTHALAMIC INJECTION OF α-MSH. D. I. Diz* and D. M. Jacobowitz. Lab. of Clin. Science, NIMH and NIGMS, Bethesda, MD 20205.

In an attempt to define possible actions of brain α-melanocyte stimulating hormone (α-MSH), the effect of discrete intrahypothalamic injections of α-MSH on blood pressure and heart rate was studied. Under light halothane anesthesia pre-injection blood pressure and heart rate of 48 Sprague-Dawley rats was 93 ± 1 mm Hg and 352 ± 6 bpm, respectively. Microinjections of 0.5 - 1.5 nmol α-MSH or phosphate-buffered saline vehicle were given in volumes of 100 - 300 nl via double-barrelled glass micropipettes (O.D. 30-70 µm). Both sides of the brain in each rat were used. Injection of α-MSH into the rostral dorsomedial nucleus (A4900 - 4600; König and Klippel) resulted in a 13% increase in heart rate (43 ± 6 bpm; n = 22) which was characterized by a gradual onset with the peak response occurring at 7 ± 1 min and a duration of 51 ± 6 min. A slight increase in blood pressure was also observed (5 ± 1 mm Hg). Tachyphylaxis to the heart rate response was apparent after repeated injections of α-MSH into the same site. Injection of vehicle into the same site (either before or after α-MSH) had no effect on blood pressure or heart rate. In contrast to the increase in heart rate observed following α-MSH injection into the rostral dorsomedial nucleus, injections of α-MSH or vehicle into the medial preoptic nucleus, anterior, paraventricular or posterior hypothalamic nuclei had minimal effects on blood pressure and heart rate. In addition, up to ten times the intrahypothalamic dose of α-MSH was injected into the lateral cerebral ventricle or intravenously without effect (3 rats). Since previous immunohistochemical studies have demonstrated high densities of α-MSH-containing nerve fibers in the dorsomedial nucleus, our data suggest a possible physiological involvement of α-MSH in central control of heart rate.

281.12 HYPOTHALAMIC KNIFE CUTS ATTENUATE HYPERGLYCEMIA AND BLOCK HYPOTHERMIA FOLLOWING INTRACISTERNAL BOMBESIN IN RATS. M.W. Gunion*, C.V. Grijalva, Y. Tache, and D. Novin. Center for Ulcer Research and Education and Dept. of Psychol., UCLA, Los Angeles, CA 90024.

Centrally administered bombesin (B) has a number of effects on functions regulated by the autonomic nervous system. We examined the neural mechanisms of two of bombesin's effects: hyperglycemia, and poikilothermia (leading to hypothermia at normal room temperature). Singly housed male hooded rats (208-272 g) were deprived of food but not water for 24 hr prior to the experiment. They were anesthetized (methohexital, 58 mg/kg ip), rectal temperatures taken, and given one of 5 bilateral surgical treatments: lateral hypothalamic parasagittal wire knife cuts (3 mm) along the medial edge of the internal capsule (LAT) or in the plane of the fornix (MED), coronal knife cuts (1 mm) at the anterior (ANT) or posterior (POST) border of the lateral hypothalamus (LH), or control surgery (CON) consisting of normal guide shaft penetration without wire extrusion. Immediately after the cuts B (500 ng) or saline (S) (both 10 µL) was injected into the cisterna magna. Two hr later temperatures were taken again and the rats, all conscious, were decapitated.

CON-B rats showed pronounced hyperglycemia as expected (116 mg/dl greater than CON-S, $p<.01$). Attenuated hyperglycemia was seen in the ANT-B and MED-B groups (59 and 77 mg/dl above appropriate S groups, $p<.05$ and $p<.01$ respectively). Glucose was elevated nonsignificantly in the LAT-B and POST-B groups (39 and 33 mg/dl above appropriate S groups, both $p>.05$). None of the cuts altered glucose levels in S rats.

B suppressed rectal temperature in CON rats as expected (-2.2 deg compared to CON-S, $p<.01$) and also in MED rats (-2.6 deg compared to MED-S, $p<.01$). In contrast, LAT-B rats did not show hypothermia, and actually had slightly higher temperatures than LAT-S rats (+0.3 deg, $p>.05$); LAT cuts had no effect on temperature by themselves (CON-S vs. LAT-S, $p>.05$). B induced hypothermia was greatly attenuated in ANT and POST groups; in fact, it was not significant (ANT=-1.3 deg, POST=-0.6 deg, both $p>.05$). Only ANT cuts caused a significant elevation of temperature (+1.8 deg compared to CON-S, $p<.05$), although MED and POST cuts caused nonsignificant increases (+1.4 and +1.0 deg compared to CON-S, both $p>.05$).

These results suggest that fibers passing the lateral and posterior borders of the LH are involved in the hyperglycemic response to B but are not the sole substrate(s) for this effect. They also suggest that fibers crossing the lateral LH border are critical for the B disruption of homeothermia. (Supported by AM 30110 to Y.T. and NS 7687 to D.N.)

281.13 LATERAL HYPOTHALAMIC LESIONS BLOCK THE HYPERGLYCEMIC RESPONSE INDUCED BY INTRACISTERNAL BOMBESIN IN RATS. C.V. Grijalva, M.W. Gunion*, Y. Tache, and D. Novin. Dept. of Psychol. and Brain Research Institute, UCLA, Los Angeles, CA 90024, and Pediatric Research Institute, Hospital St. Justine, Univ. of Montreal, Montreal, Quebec, Canada H3T 1C5.

Intracerebroventricular administration of bombesin in rats inhibits gastric acid secretion (Tache et al., Pro. Natl. Acad. Sci. 77:5515, 1980; Gastroenterology 81:298, 1981) and also induces hyperglycemia, hyperglucagonemia and relative or absolute hypoinsulinemia (Brown et al., Life Sci. 21:1729, 1977: Endocrinology 105:660, 1979). We recently reported that lateral hypothalamic (LH) lesions prevented the increase in gastric pH and plasma gastrin levels induced by intracisternal injections of bombesin. In the present study we further examined the possibility that LH lesions alter the effects of bombesin on blood glucose and free fatty acids (FFA).

Groups of rats were food deprived for 24h, anesthetized with methohexital (50 mg/kg, ip) and then given bilateral electrolytic lesions of the LH (n=12), lateral thalamic nuclei (LT, n=6), or sham operations (n=10). Immediately thereafter, rats from each group were injected intracisternally with either bombesin (500 ng: LH group, n=6; LT group, n=4; sham group, n=5) or saline. All rats were decapitated 2h later and their trunk blood was assayed for glucose and FFA.

Saline-treated groups exhibited similar blood glucose values. Bombesin injections induced a significant increase in blood glucose levels in both the LT group and sham group, but not in the LH group.

There was no significant difference in FFA levels between saline-treated and bombesin-treated rats regardless of group. However, FFA levels were lower in the LH rats when compared to the sham animals.

These results indicate that the LH area is not only important in influencing the action of bombesin on gastric secretory and gastrin functions, but also may mediate some of bombesin's effect on glycemia by altering sympathetic tone (Brown et al., Endocrinology 105:660, 1979).

Supported by NIH Grant NS 7687.

281.14 PHARMACOLOGICAL AND PHYSIOLOGICAL MANIPULATIONS ON THE RELEASE OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) AND CHOLECYSTOKININ (CCK) FROM CAT CEREBRAL CORTEX AND VENTRICLES IN VIVO. J.-Y. Wang*, T.L. Yaksh* and V.L.W. Go* (SPON: E.H. Lambert). Depts. of Pharmacology, Neurosurgical Research and Gastroenterology, Mayo Foundation, Rochester, MN 55905.

VIP- and CCK-immunoreactive cell bodies have been observed in various columnar layers in cerebral cortex, the terminals of these peptide-containing neurons are located in superficial lamina I. The cell bodies and terminals of VIP- and CCK-containing neurons are also rich in periventricular regions. In view of the fact that the two peptides are present in cerebrospinal fluid (CSF) and are releasable in vitro, we have employed two in vivo preparations of the cat to study the release of VIP and CCK from terminal areas: 1) cortical cups (two 1-cm diameter plastic cylinders were agar-sealed on the pial surface after bilateral craniotomy and reflection of the dura); 2) ventriculo-cisternal superfusion (a 22 ga inflow cannula inserted into lateral ventricle stereotaxically and an outflow cannula placed in the cisterna magna). Superfusions were carried out at a rate of 100-200 µl/min with artificial CSF containing albumin/bacitracin. Superfusate samples were collected consecutively at 30-min intervals, lyophilized and reconstituted to 1/3 volume for radioimmunoassay. Resting levels of VIP- and CCK-immunoreactivity were 13.79 ± 4.06 and 10.7 ± 2.46 fmol/30 min/cm^2 cortex in cortical superfusate; 31.62 ± 4.99 fmol and 25.62 ± 4.44 fmol/30 min in ventricular superfusate, respectively. The effects of the addition of several pharmacological agents are summarized as follows in terms of % increase above baseline (↑%).

	Cortex (↑%)		Ventricles (↑%)	
	VIP	CCK	VIP	CCK
K+ (50 mM)	215 ± 26	232 ± 52	180 ± 19	261 ± 35
Veratridine (10^{-5}M)	230 ± 75	270 ± 52	354 ± 52	640 ± 167
Picrotoxin (10^{-4}M)	160 ± 37	328 ± 56	408 ± 73	523 ± 61
Kainic acid (10^{-6}M)	192 ± 178	101 ± 196	387 ± 62	449 ± 121
NE (10^{-4}M)	N.S.	N.S.	N.S.	N.S.
DA (10^{-4}M)	N.S.	N.S.	N.S.	↓*
Carbachol (10^{-4}M)	N.S.	N.S.	↓*	↓*

N.S. = no significant change.
* = decreased but not statistically significant.

The K+-evoked but not basal release of both peptides was inhibited by the substitution of Co++ (4 mM) for calcium in artificial CSF. Bilateral electrical stimulation of the mesencephalic reticular formation increased peptide levels in some experiments. Gel filtration chromatography (Sephadex G-50, superfine) showed that the VIP-immunoreactivity in basal and evoked superfusate co-chromatographed with authentic VIP-28 while immunoreactive CCK was isographic with CCK-8. (Supported by NS 16541, AM 07198-06 and Mayo Foundation.)

281.15 CHANGES IN DISTRIBUTION OF SUBSTANCE P-LIKE COMPOUNDS AND BLOOD PRESSURE IN RATS FOLLOWING HYPOPHYSECTOMY, BILATERAL SPLANCHNICECTOMY, AND PREGNANCY. M. L. Swenberg*, H. Tanase*, and W. Lovenberg.* (SPON: D. L. Livengood). Section on Biochem. Pharmacol., Natl. Heart, Lung, and Blood Inst., N.I.H., Bethesda, MD 20205.

The neuroendocrine system of Sprague-Dawley rats (250 ± 50 gm) was manipulated in various ways (hypophysectomy, bilateral splanchnicectomy and pregnancy). In these animals and their appropriate control groups, the growth, blood pressure, and substance P content of adrenal, kidney and central nervous systems were measured. Specific brain regions analyzed were: hypophysis, hypothalamus, substantia nigra (SN) and caudate-putamen (CP).

Blood pressure (BP) was measured by a tail-cuff method; SP like compound was determined by radioimmunoassay (RIA) with antisera raised in New Zealand white rabbits that were injected subcutaneously with SP coupled to bovine albumin (BSA-SP). SP was iodinated with Bolton Hunters reagent.

Growth in all operated animals was slightly inhibited, and completely stopped in those hypophysectomized. The only blood pressure (BP) change observed was the decrease in the hypophysectomized animals, however significant changes in SP distribution were observed in various groups. Bilateral splanchnic nerve transection slightly increased SP in both adrenal and kidney, but decreased SP in all brain regions studied (25-50%). Hypophysectomy increased SP in hypothalamus but decreased SP in SN. Cortisone administration returned the SP level to normal in SN of hypophysectomized rats. This result indicates the possibility that corticoid hormone may be partially responsible for the regulation of SP levels in SN.

Pregnancy tended to increase SP in most organs and tissues examined. However there was also a dramatic increase in SP concentration in the kidneys and blood of pregnant females.

The current data suggest that the neuroendocrine system has an important impact on SP in both central and peripheral SP systems. It is possible that corticoids influence the synthesis of SP both centrally and in the periphery and SP may play an important role in pregnancy.

281.16 SUBCUTANEOUS HYPERTONIC SALINE ASSAY AS A MEASURE OF ANALGESIA FOLLOWING INTRATHECAL NEUROTENSIN, OPIOIDS AND MONOAMINES IN MICE: CORRELATION WITH INHIBITION OF SUBSTANCE P-INDUCED BEHAVIOR. Janice L. K. Hylden and George L. Wilcox. Department of Pharmacology, Univ. of MN, Mpls., MN 55455.

We have recently been interested in the action of endogenously-occuring antinociceptive monoamines and peptides at the level of the spinal dorsal horn. Two analgesic assays were employed to determine whether intrathecally (i.t.) injected compounds were acting pre- or postsynaptically with respect to primary afferent fibers 1) subcutaneous hypertonic saline assay (s.c. NaCl) and 2) i.t. substance P assay (SP). In the first assay, mice were given 0.2 ml 4% NaCl s.c. on the lower abdomen 5 min after or 30 sec before i.t. injection. The number of licking or scratching motions directed toward the s.c. injection site was then counted for the 2 or 3 min. following s.c. NaCl. The SP assay consisted of coadministering the compound with 10ng SP i.t. and counting the licking and scratching motions directed toward the abdomen for 1 min. immediately following injection. Neurotensin (NRT) produced a dose-related inhibtion of the response to NaCl (10-100 pmol), but did not significantly alter the SP-induced behaviors in this dose range. Norepinephrine (NE), on the other hand, produced potent analgesia in both assays (ED_{50}s:200pmol NaCl assay and 40pmol SP assay). Morphine (M) and the endogeous opioids Leu-enkephalin (LE) and Met-enkephalin (ME) were also active in both assays (ED_{50}s:NaCl assay 2.4, 1.8 and 1.0 nmol; SP assay 0.5, 1.2 and 0.1 nmol for M, LE and ME respectively). The data indicate that NRT acts primarily presynaptically under these conditions, that is it can block the response to peripheral stimulation but not to exogenously applied SP (primary afferent nociceptor transmitter). NE and the opioids tested blocked both responses suggesting a significant postsynaptic site of action for these compounds. (Supported by USPHS Grant DA01933, T32GM07397 and RR05385.)

281.17 EFFECTS OF INTRASPINALLY INJECTED SUBSTANCE P ANTAGONISTS ON BEHAVIORAL RESPONSES TO NOXIOUS STIMULATION OF INFLAMED PAWS IN MICE. F.J.Einspahr* and M.F.Piercey, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The analgesic activities of substance P antagonists, enkephalins, and opioid analgesics were determined by an adaptation of the method of Randall and Selitto (Arch. int. Pharmacodyn., 1957, CXI, No. 4, p. 409) using mice. A hind paw, inflamed by a subcutaneous injection of carrageenan, was pinched at steadily increasing pressures until the mouse tried to escape or oriented toward the foot. Response thresholds were increased in a dose-related manner by intraperitoneal injections of the opioids ethylketocyclazocine, U-50,488H, morphine, and pentazocine. The weak analgesic activities if ibuprofen, aspirin and tolectin were detected 15 minutes after intraperitoneal dosage, indicating the sensitivity of the assay, yet tranquilizing doses of chlorpromazine did not change pain thresholds from control. Opioids were especially potent in elevating thresholds when injected intraspinally as were the enkephalins, dynorphin and FK-33824 (D-Ala^2, N-Me-Phe^4, Met-(O)-Ol enkephalin). The substance P antagonists, D-Pro^2, D-Phe^7, D-Trp^9 SP and D-Pro^{2}DTrp^{7,9} SP (Science 214:1361, 1981) were weakly analgesic when injected intraspinally. These results support earlier suggestions that SP antagonists represent a potentially new class of analgesics. However, currently available SP antagonists appear to have weaker analgesic efficacy than opioid analgesics.

281.18 IN VITRO AND IN VIVO STUDIES OF SUBSTANCE P (SP) AND TWO PUTATIVE SP ANTAGONISTS. E.R. Baizman*, D.M. LoPresti*, N.J. Meo*, A.K. Pierson* and B.A. Morgan* (SPON: F. Rice). Sterling-Winthrop Research Institute, Rensselaer, NY 12144.

SP is believed to play a physiological role in nociceptive sensory transmission or modulation, particularly in the spinal cord and brainstem. This concept has been strengthened by recent reports on the actions of D-Pro^2, D-Phe^7, D-Trp^9-SP (PFW) and D-Pro^2, D-Trp^{7,9}-SP (PWW), two putative SP antagonists which block the effects of exogenous SP in vitro (Folkers, et al, Acta. Physiol. Scand. 111:505, 1981) and in vivo (Piercey, et al. Science 214:1361, 1981). In our experiments using the atropinized guinea pig ileum longitudinal muscle, both undecapeptides evoked some SP-like agonist activity (0.001 x SP); as antagonists, they produced parallel shifts in the SP dose-response curve only in concentrations >10μM (pA_2 for PFW=4.6; pA_2 for PWW=5.5). Capsaicin (0.01-1.0 μM) evoked prolonged contractions of the ileal muscle which were abolished in preparations desensitized to the contractile action of SP. These sustained capsaicin-induced contractions showed immediate reversal after addition to the bath of 10^{-5}M of either PFW or PWW.

In in vivo studies, the behavioral syndrome elicited in mice by intrathecal (i.t.) injection of 22 nmoles of SP (Hylden and Wilcox, Brain Res., 217:212, 1981) was antagonized by coadministration or 5-min. pretreatment with PFW (ED_{50}=0.8 nmole) or PWW (ED_{50}=1.0 nmole). In contrast to earlier reports, we found that i.t. morphine pretreatment (ED_{50}=0.9 nmole) also produced naloxone-reversible blockade of the SP-behavioral syndrome. Naloxone (1 and 10 mg/kg s.c.) was ineffective in reversing actions of the SP antagonists. Writhing behavior in mice induced by acetylcholine (3.2 mg/kg i.p.) was antagonized by 5-min i.t. pretreatment with PFW (ED_{50}=1.7 nmoles), PWW (ED_{50}=1.6 nmoles) and by morphine (ED_{50}=1.4 pmoles). We and others (Piercey, et al., ibid, 1981) have noted hindlimb flaccidity at the highest intraspinal dose of either peptide (6.5-6.8 nmoles), although inhibition of writhing was observed at lower doses, in the absence of overt motor deficits. These experiments support the concept that antagonism of the apparent postsynaptic actions of exogenous SP, initially confirmed in vitro, results in attenuation of in vivo perception of a noxious chemogenic stimulus. The models may be utilized to examine novel SP antagonist candidates as potential non-opioid antinociceptive agents.

281.19 SUBSTANCE P PROTECTS AGAINST NICOTINIC DESENSITIZATION OF CULTURED ADRENAL CHROMAFFIN CELLS. P. Boksa* and B.G. Livett (SPON:R.Capek). Division of Neurology, Montreal General Hospital and McGill University, Montreal, Canada.

Studies on the neuromuscular junction have shown that certain drugs (e.g. d-tubocurarine) which inhibit nicotinic receptor activation also affect nicotinic receptor desensitization. We have previously shown that substance P (SP), a peptide endogenous to the splanchnic nerve, inhibits the ACh- or nicotine-induced release of catecholamines (CAs) from adrenal chromaffin cells. Therefore, in the present study we have examined whether SP also affects desensitization of the nicotinic response in cultured adrenal chromaffin cells. Chromaffin cells were isolated by retrograde perfusion of bovine adrenal medullae with collagenase, the cells purified on Percoll, and maintained as monolayer cultures as described previously (Livett, B.G. et al, Nature 278: 256, 1977). On the day of the experiment, cultures were loaded with [^{3}H]l-norepinephrine ([^{3}H] NE, 10^{-7}M). Following this, the cultures were pre-incubated with desensitizing concentrations ($5x10^{-4}$-$5x10^{-3}$M) of nicotine, washed, and then re-stimulated with a concentration of nicotine ($7.5x10^{-6}$M) that produces a near maximal release of CAs; [^{3}H] NE release during the re-stimulation period was measured. Pre-incubation with a high [nicotine] for 5 min caused a 30-50% decrease in [^{3}H] NE release during re-stimulation compared to control values (no pre-incubation); this decrease in NE release is referred to as "desensitization". Desensitization was not due to depletion of [^{3}H] NE stores during pre-incubation with a high [nicotine] since cultures pre-incubated with high [nicotine] in a 0Ca++ medium (where release is blocked) were also desensitized; this experiment also demonstrates that desensitization of CA release by nicotine is not a Ca++-dependent process. Cultures pre-incubated with a high [nicotine] in the presence of SP (10^{-5}M) released amounts of [^{3}H] NE during re-stimulation equal to that released by control cultures (no pre-incubation); thus SP protected against desensitization of CA release by nicotine. The ED_{50}'s for SP for protection against desensitization (10^{-6}M) and for inhibition of nicotine-induced CA release ($3x10^{-6}$M) were similar. d-tubocurarine (ED_{50} = 10^{-5}M) also protected against desensitization of CA release by nicotine (10^{-3}M) in cultured adrenal chromaffin cells.

The observation that SP protects against nicotinic desensitization of CA release in cultured adrenal chromaffin cells suggests a similar role for SP in vivo. During stress conditions when rapid firing of the splanchnic nerve releases large amounts of ACh on to adrenal chromaffin cells, simultaneous release of SP may ensure a prolonged release of CAs by protecting against rapid desensitization of the ACh receptor.

(Supported by Canadian MRC)

281.20 PHARMACOLOGIC MANIPULATIONS OF PEPTIDE-MEDIATED TRANSMISSION IN RAT SUBSTANTIA NIGRA: ANTICONVULSANT EFFECTS. D.S. Garant*, M.J. Iadarola[1], and K. Gale (SPON: F.G. Standaert). Georgetown Univ. Sch. of Med. and Dent., and [1]NIMH, St. Elizabeths Hospital, Washington, D.C.

We have previously demonstrated that inhibition of outflow from substantia nigra (SN) confers protection from experimental seizures in rats. Intranigral microinjections of directly- and indirectly-acting GABA agonists protect against seizures induced by maximal electroshock (MES) or chemoconvulsants (Iadarola and Gale, Soc. Neurosci. Abstr.# 188.16,1981). Moreover, discrete electrolytic or kainate lesions of SN significantly attenuate bicuculline-induced seizures (Garant, et al, Fed. Proc. 41, 1064, 1982). More recently, we have found that bilateral electrolytic lesions of SN protect rats from tonic hindlimb extension (THE) in the MES test as well. These results suggest that suppression of nigral efferent activity may be an important mechanism for reducing susceptibility to generalized convulsions. We have therefore extended our observations to include manipulations of peptidergic systems in SN which may influence the excitability of nigral efferents.

As substance P (SP) has been reported to mediate an important excitatory input to SN, and as opiate receptor activation has been associated with inhibition of SN outflow, the SP antagonist [d-Pro-^{2},d-Phe-^{7},d-Trp-^{9}]-SP and the opiate agonist morphine were evaluated for anticonvulsant activity. Rats received stereotaxically placed microinjections of either compound and were evaluated in the MES test 3 hr later. 10-20ug of the SP antagonist placed bilaterally into SN resulted in an 80% protection from THE; 20ug morphine sulfate into each SN reduced THE duration by 55%. The bilateral injections of both compounds into SN caused sniffing and gnawing stereotyped behavior which persisted for 2-2.5 hr. Unilateral microinjections of these compounds into SN produced contraversive turning behavior but did not protect against seizures.

Based on our preliminary evidence, it appears that anticonvulsant effects may be obtained by manipulating any one of a number of transmitter candidates in the SN. We are currently investigating intranigral application of other peptide analogs with agonist and antagonist activity in terms of their effects on experimental seizures.

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281.21 EFFECTS OF ATRIAL GLAND PEPTIDES ON BAG CELLS AND OVOTESTIS IN INTACT APLYSIA. G.T. Nagle, S.D. Painter and J.E. Blankenship. Marine Biomedical Inst., Univ. Tx. Med. Br., Galveston, TX 77550.

When crude extracts of homogenized A. californica atrial gland (AG) are injected into Aplysia, animals lay eggs within 120 min (Arch et al., J. Comp. Physiol. 128:67, 1978). The atrial gland contains at least 3 peptides (A,B and ERH (egg releasing hormone)) that trigger the bag cells (BC) to release egg laying hormone (ELH) in vitro and cause egg laying in vivo. The ability of AG peptides to trigger the BC in vivo has not been shown, but is a necessary prerequisite for understanding the role of these peptides in the regulation of egg laying.BC activity was monitored in 5 animals in vivo with cuff electrodes (Pinsker & Dudek, Science 197:490, 1977). We found that the ovotestis and BC are differentially sensitive to AG extract injections. In 7 experiments injections of ≤.03 AG did not trigger BC discharges (BCD), yet egg laying occurred with a latency of 37-85 min. In 9 cases, doses of .01-.10AG caused egg laying with a similar latency, but also triggered a BCD. In contrast to studies of spontaneous egg laying in intact Aplysia (Dudek et al., J. Neurophysiol. 42:804, 1979), there was no correlation between time of onset of BCD and onset of egg laying when AG extracts are given (e.g., in 3 of the 9 experiments a BCD did not occur until 8-22 min after egg laying had begun). Latency of BCD was correlated to AG extract concentration in a dose-dependent manner. In all 5 animals, the BC were less sensitive to AG extracts than the ovotestis. When egg laying episodes occur without BCD, it is most likely that ERH is acting on the ovotestis to cause egg laying because of this peptide's structural homology to ELH, and because ERH causes egg laying in animals whose BC have been removed.

We have begun to look for regions in the Aplysia CNS where AG peptides might act to trigger BCD. In vitro lesion and ablation studies show that an intact neural pathway between the site of extract application and the BC is required. Extracts induce BCD when selectively applied to the cerebral ganglion, pleural ganglia, cerebropleural connectives or the rostral 1 cm of the pleurovisceral connective (PVC), but not when applied to the pedal or buccal ganglia. In vivo recording experiments confirm these results. When PVCs are cut midway between the pleural and abdominal ganglia, injections of AG extracts only rarely trigger BCD (1 of 5 animals). In contrast, when the PVCs are severed just caudal to the pleural ganglia, BCD consistently follow AG extract injection (N=4 animals). (Supported by NSF PCM 79-12175, NIH NS 11255, NS 07025 and NS 07010).

281.22 DIFFERENTIAL DOSE RESPONSE CHARACTERISTICS OF INTERFERON IN VENTROMEDIAL HYPOTHALAMUS AND HIPPOCAMPUS. B. Prieto-Gomez*, C. Reyes-Vazquez and N. Dafny. Department of Neurobiology, School of Medicine, University of Texas, Houston 77025 and Departamento de Fisiologia, Facultad de Medicina, UNAM, Mexico.

It has been shown that interferon (IF) applied to cerebral and cerebellar neurons in culture produces an increase in the spontaneous and evoked activity. This effect is different from those resported for excitatory substances. The objective of this study was to determine the effect of the microiontophoretic application of three different doses of IF on two specific CNS sites, i.e., the ventromedial hypothalamus (VMH) and dorsal hippocampus (HIPP). Urethane anesthetized Sprague-Dawley rats and an array of 4 micropipettes were used and placed in VMH or HIPP. The micropipettes contained: (1) recombinant leukocyte A IF (Hoffmann-La Roche), (2) L-glutamate (GLUT), (3) fast green in NaCl 4 M (balance and test current) and (4) the recording electrode filled with Na Cl 4 M and glued alongside the multibarrel. Three different doses (20, 50 and 100 nA) of IF were applied on 15 cells in the HIPP and on 15 cells in VMH. The recording session for each cell consisted of 60 sec of spontaneous activity (control period) followed by 60 sec of drug application and 140 sec of post-injection activity. Three min of recovery time were permitted between each recording session. GLUT and current effects were tested in each cell. All the IF doses caused an increase in the spontaneous activity in all the HIPP neurons with dose response characteristics. However, the VMH units responded to IF injection mainly (60%) by a decrease in their activity, some (30%) failed to respond and in a few cells (10%) IF caused an increase in their electrical discharges. GLUT produced an increase in both sites as expected and current injection had no effect. Our results suggest that IF has site specific effects since it exhibits dose response characteristics and had different effects in the two structures studied.

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281.23 DISSOCIATION OF THE ACTIONS OF CAPSAICIN ON DEPLETION AND RELEASE OF SUBSTANCE P. J.H. Selsky* and C.J. Helke, Dept of Pharmacology, Uniformed Services Univ of the Health Sciences, Bethesda, MD 20814

Acute administration of capsaicin (CAP) evokes the release of substance P (SP) from nuclei which receive primary afferent SP innervation, i.e. the dorsal horn of the spinal cord (DH), the trigeminal nucleus (NTV), and the nucleus tractus solitarius (NTS) (Life Sci., 29:1779, 1981 and 25: 629, 1979). Chronic administration of CAP to neonates depletes SP in the DH and NTV whereas no reduction was found in the NTS of the adult animals (Brain Res. 222:428, 1981). These data suggested that there is no correlation between the ability of CAP to evoke SP release and to deplete SP content. To test this, we performed 2 series of experiments. First, because CAP administration to neonates produced morphological changes in the NTS (J. Comp. Neurol. 190: 781, 1980), different modes of drug administration were used to confirm the inability of CAP to cause SP depletion in the NTS. Second, we assessed the ability of CAP to evoke SP release in vitro from NTV tissue of adult rats which were treated with CAP as neonates.

In the first series of experiments, CAP was administered to adult rats either subcutaneously (50, 100, 200, 200 & 400 mg/kg on 5 consecutive d) or intraventricularly (30 or 60 µg). Seven to 9 d later the rats were decapitated, CNS tissues microdissected and assayed for SP content by RIA. A significant depletion of SP content of the NTV (56-74%) was detected in both groups, whereas no change was found in the SP content in the NTS.

Because the CAP-induced morphological changes in the NTS were seen in neonatal rats killed 1 d after treatment with CAP (50mg/kg s.c.), we mimicked these conditions and assayed SP in the NTS and NTV of the neonatal rats. Again we found a depletion of SP in the NTV and no change in the NTS. We conclude that CAP does not deplete SP from the NTS, whereas it can evoke the release of SP from the NTS.

In the second series of experiments, rats were treated with CAP (50mg/kg s.c.) as neonates and sacrificed at 3 mo of age. The NTV was dissected and SP release was stimulated in vitro with either 50mM K+ or 33 µM CAP. The endogenous SP released was assayed by RIA. Tissue content and amount of SP released (pg/mg protein) by either K+ or CAP were significantly reduced in the animals chronically treated with CAP. However, CAP and K+ did stimulate SP release from both groups and when release was expressed as percent of endogenous SP content, the values were similar between CAP treated and the vehicle control groups. Therefore, although CAP pretreatment reduced the SP content of the NTV a similar percent of the pool was available for evoked release in CAP-treated and vehicle control rats.

These data suggest a dissociation of the actions of CAP on the depletion and the release of SP. Supported by USPHS Grant HL-26849.

282.1 GABA IN THE ANTENNAL LOBES OF METAMORPHOSING AND MATURE *MANDUCA SEXTA*. T. G. Kingan and J. G. Hildebrand, Dept. of Biological Sciences, Columbia University, New York, N. Y. 10027.

Previous biochemical observations in our laboratory (Maxwell *et al., Comp. Biochem. Physiol. 61C*: 109-119, 1978) suggest that γ-aminobutyric acid (GABA) may function as a neurotransmitter in the antennal lobe (AL) of the moth *Manduca sexta*. Thus, incubation of brains *in vitro* with [^{3}H]glutamic acid leads to accumulation of [^{3}H]GABA in the ALs. Because GABA serves widely in invertebrates as an inhibitory neurotransmitter and because intracellular recording from AL interneurons reveals much inhibitory synaptic activity (Matsumoto and Hildebrand, *Proc. Roy. Soc. B215:* 249-277, 1981), we hypothesize moreover that GABA is an inhibitory neurotransmitter employed by at least some interneurons in the AL.

To extend these studies of putative neurotransmitters in the AL, we have used high-performance liquid chromatography (HPLC) for microanalysis of amino acids, including GABA, in AL extracts. Tissue was rapidly dissected and extracted in acetone/1N formic acid (85/15 v/v). Extracts were fractionated on a DC-5A cation-exchange column (Dionex) with sodium citrate and sodium phosphate buffers, and eluted amino acids were derivatized with *o*-phthalaldehyde and quantified by fluorometry.

The amino-acid profiles of adult ALs and antennal nerves (which comprise antennal-sensory afferent axons) were similar, except that GABA could be detected only in the AL extracts. Studies of ALs taken at various stages in the 18-day period of metamorphic adult development showed that GABA is detectable at least by day 6 (approximately 30% of adult development) and increases through eclosion of the adult moth. Levels estimated were: *day 6*, 20-30 pmol/AL; *day 11*, 80 pmol/AL; *day 16*, 150 pmol/AL; and *2 days post-eclosion*, 300 pmol/AL. To investigate the role of afferent inputs on this development of GABA levels, we deprived the AL of antennal afferent inputs throughout adult development by excising one antennal imaginal disk in 5th-instar larvae, leaving the contralateral antennal pathway as an unoperated control. The deafferented AL, examined 2 days after eclosion of the adult, yielded 55-68% of the amount of GABA found in the control AL. This finding suggests that many or all GABA-containing AL neurons survive and develop the biochemical machinery for synthesis and storage of GABA despite the chronic absence of normal afferent inputs.

These studies have been supported by U.S. Army contract DAAG29-81-K-0091 and NSF grant BNS 80-13511, as well as an NIH postdoctoral research fellowship awarded to T.K.

282.2 EVIDENCE FOR GLUTAMATE AS A CENTRAL EXCITATORY NEUROTRANSMITTER IN INSECTS. Sompong Sombati* (SPON: S. Zill). Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

Glutamate is considered to be the transmitter at excitatory neuromuscular junctions in insects. According to Dale's principle, the transmitter at central synapses of the same excitatory motorneurons should also be glutamate. However, glutamate had not previously been shown to have an excitatory effect within the insect central nervous system. This report shows that glutamate has a central excitatory action and strongly suggests that it is the neurotransmitter in an identified central pathway.

In the locust, Schistocerca gregaria, and the grasshopper, Schistocerca americana, there is a monosynaptic excitatory pathway from the fast extensor tibiae (FETi) to the posterior fast flexor tibiae (PFFl) motorneurons. The effects of bath application of glutamate, and also of a glutamate receptor antagonist, upon this central synapse were examined. In the isolated ganglion, simultaneous intracellular recordings from both cells during bath application of the glutamate antagonist, glutamate diethyl ester (GDEE), showed that the post-synaptic response in PFFl to spikes in FETi was reversibly blocked by as much as 75% of the original value after 2 h. Bath application of glutamate caused depolarization of PFFl, followed by desensitization. The membrane potential returned to near its resting value in about 20 s, after which spikes from FETi failed at first to elicit psp's in PFFl. Psp's returned following extensive washing.

Glutamate was iontophoresed through a microelectrode placed near the neuropilar processes of these motorneurons. Spikes were elicited in PFFl, followed by desensitization. The sites sensitive to glutamate were highly localized; moving the glutamate electrode 10 microns away from a sensitive spot could result in loss of response. Bath application and iontophoretically-applied glutamate also excited FETi and were followed by desensitization.

These results show that the pathway between FETi and PFFl is probably glutamatergic and suggest that glutamatergic transmission may be a widespread phenomenon in the insect central nervous system.

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282.3 GAMMA-GLUTAMYLHISTAMINE (γ-GHA) IS AN ENDOGENOUS CONSTITUTENT OF APLYSIA NERVOUS TISSUE. C. Stein and D. Weinreich. Dept. of Pharmacology and Experimental Therapeutics, University of Maryland, School of Maryland, Baltimore, MD 21201.

Radiolabeled histamine (HA) is metabolized to γ-GHA in both vertebrate and invertebrate nervous tissue. In rat brain, < .001% of labeled HA, administered intraventricularily is converted to this peptido-amine (Konish and Kakimoto, 1976). In constrast, the CNS of Aplysia metabolizes > 90% of labeled HA taken up by ganglia in situ to γ-GHA (Weinreich, 1979).

Our interest in the γ-glutamylation of HA derives from continued efforts to provide evidence for histaminergic synaptic transmission. Previous studies in Aplysia ganglia and neurons have characterized the presence of a new enzyme, γ-GHA synthetase, which catalyzes the synthesis of γ-GHA from glutamate and HA (Stein and Weinreich, 1982). In this study we have quantitated the endogenous levels of γ-GHA in various tissues of Aplysia.

Formic acid/acetone supernatant fractions of pooled ganglia and other tissues were fractionated by reverse-phase HPLC to separate endogenous γ-GHA from HA. Eluates having retention times of standard. γ-GHA (in the presence or absence of tissue) were collected. HA released from γ-GHA following acid hydrolysis was quantitated by chemical analysis.

The Aplysia CNS contains about 9.1 pmole γ-GHA, about 1/70th the endogenous HA content. This peptide was detectable in all five major ganglia - the abdominal (7.0 pmole/mg protien) was highest while the pedal ganglion (2.8 pmol/mg protein) contained the lowest levels. Endogenous γ-GHA was also measurable in connective tissue, penis muscle, and buccal muscle (2.5, 1.3 and .4 pmole/mg protein, respectively). Heart and "liver" did not possess dectable levels of γ-GHA (< .1 pmole/mg protein).

These findings support our working hypothesis that γ-glutamylation represents a physiologically relevant pathway of HA metabolism, perhaps participating in the inactivation of neuronnaly released HA. The presence of γ-GHA in tissues of Aplysia californica represents the first instance where this imadizole-peptide has been found endogenously. (Supported by NSF grant BNS-8113552 to D.W.)

282.4 DETERMINATION OF NEUROTRANSMITTERS IN THE ANTERIOR AORTA OF APLYSIA S.L. Knock* and D.J. McAdoo, Marine Biomedical Institute and Human Biological Chemistry and Genetics, UTMB, Galveston, Texas 77550

Potential neurotransmitter and neuromodulator substances present in the anterior aorta of Aplysia were identified and quantified. Four neurons, whose cell bodies are found in the abdominal ganglion, are known to innervate the anterior aorta. Previous experiments have provided evidence that the two RD_{AAI} neurons are cholinergic and inhibitory, the RD_{AAE} neuron is serotonergic and excitatory and that the R_{14} cell utilizes glycine to modulate the excitatory input to the anterior aorta. The anterior aorta was removed from the animal and extracted for acetylcholine and biogenic amines. The white fibers innervating the anterior aorta which contain the R_{14} processes, were dissected from surrounding tissue and extracted for amino acid analysis. Acetylcholine was detected in the amount of 1.0 ± 0.2 nmoles Ach/mg protein in the anterior aorta extract using a radioenzymatic assay. Serotonin was also detected in this extract using High Performance Liquid Chromatography and Gas Chromatography Mass Spectrometry analysis. The amount of serotonin detected by High Performance Liquid Chromatography was 2.17 ng/mg anterior aorta protein using a citrate disodium phosphate buffer system and was 70 + 30 ng/g wet wt of aorta using an ammonium acetate buffer and trapping column. The amount of serotonin detected by gas chromatography-mass spectrometry was 2.6 ng/mg anterior aorta protein. Glycine levels determined by amino acid analysis were shown to be elevated from 10-13% (glycine/several amino acids common to all tissues) in control tissues to 45% in tissues containing R14 processes.

282.5 SEROTONIN AND OCTOPAMINE HAVE OPPOSITE MODULATORY EFFECTS ON THE CRAYFISH'S LATERAL GIANT ESCAPE REFLEX. D.L. Glanzman and F.B. Krasne, Dept. of Psychology, University of California, Los Angeles, CA, 90024.

The roles of monoamines in the central nervous system's regulation of behavior can best be understood by analyzing their effects on simple, neurophysiologically well-understood behaviors. One such behavior is the tail-flip escape response of the crayfish, Procambarus clarkii. With the aim of developing an advantageous preparation in which to study the behavioral roles of monoamines, we investigated the effects of serotonin (5-HT) and octopamine on the crayfish's escape reflex. These monoamines have been previously shown to have both central and peripheral effects in crayfish (e.g., M.S. Livingstone et al. Science 208, 76, 1980).

The monoamines, in concentrations of 10^{-3} to 10^{-6} M, were injected directly into the crayfish's ventral artery via a cannula. Axons of the sensory nerves were stimulated at rates of 1/5 sec to 1/60 sec with hook electrodes placed under second abdominal roots. The resulting excitatory post-synaptic potentials (EPSPs) in the lateral giant (LG) fibers (the command neurons for the escape response) were recorded with intracellular microelectrodes.

Serotonin depressed the size of the evoked EPSPs whereas octopamine enhanced their size. The monoamines had their major effect on the EPSP's disynaptic component, and not on its monosynaptic, electrical component. Therefore, the monoamines might be affecting the chemical synapse between the sensory afferents and the sensory interneurons. Consistent with this idea, we found that the spike threshold of an identified, first-order sensory interneuron in the reflex pathway was raised by 5-HT and lowered by octopamine. Neither monoamine significantly altered the rate at which the LG response habituated.

Behavioral roles for the monoaminergic effects reported here can be readily envisaged. Serotonin might mediate the suppressive effects of restraint upon the escape response (F.B. Krasne and J.J. Wine, J. Exp. Biol. 63, 433, 1975). On the other hand, by analogy with 5-HT's role in Aplysia's behavior (E.R. Kandel et al. Cold Spring Harbor Symp. Quant. Biol. 9, 465, 1975), octopamine might mediate dishabituation of the crayfish's escape response. Experiments are now in progress to test these possibilities.

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282.6 EFFECTS OF OCTOPAMINE AND OTHER BIOGENIC AMINES ON DEVELOPING NEUROMUSCULAR JUNCTIONS OF MANDUCA SEXTA. L. W. Klaassen* (SPON: A. E. Kammer). Kansas State Univ., Manhattan, KS 66506

Developing synapses characteristically produce postsynaptic potentials that are smaller and fatique more readily than those of mature synapses. Previous work in our laboratory demonstrated that DL-octopamine increases the amplitude of the excitatory junction potential (EJP) elicited from immature neuromuscular junctions. The purpose of this study was to examine the effect of octopamine and other biogenic amines on the ability of these junctions to respond to repeated stimulation.

Stimulating the motor nerve to the dorsal longitudinal flight muscle in pharate moths (85% developed) results in EJPs of 10 to 20 mV but does not elicit a contraction. When development is 90% complete, the amplitude of the EJP is sufficient to produce a contraction but a considerable delay is required between successive stimuli to elicit a contraction for each stimulus. As the neuromuscular junction develops, the maximum rate at which the muscle can follow each stimulus by contracting (the maximum following frequency or MMF) increases, until at 99% of development the adult frequency is reached.

Superfusion of 10^{-6} M DL-octopamine over the flight muscle of both 90% and 92% developed pharate adults results in a MMF 6.4 times greater than the MMF of untreated animals of the same age. In 95% developed moths the increase is 14-fold, and in 97% developed moths there is a 22-fold increase. At the latter age the concentration of octopamine required for an effect is 10^{-9} M. Preliminary evidence indicates that this change in the MMF is accompanied by hyperpolarization of the muscle membrane. The biogenic amines adrenaline, noradrenaline, dopamine, and tyramine did not increase the MMF of 97% developed moths at concentrations of 10^{-4} M. DL-Synephrine was as potent as octopamine; 10^{-9} M increased the MMF. Serotonin also increased the MMF but a minimum concentration of 10^{-7} M was required. The alpha-blocking agent cyproheptadine (10^{-4} M) eliminated the increased MMF caused by 10^{-7} M octopamine. Propanolol (10^{-4}) did not block the action of 10^{-7} M octopamine in increasing the MMF.

From these data and biochemical data indicating that octopamine concentrations in the blood increase late in adult development, we hypothesize that endogenous octopamine plays a significant role in preparing the flight muscle to contract in response to high frequency (20 Hz) neural output in the adult.

282.7 HETEROGENEITY OF SEROTONIN RECEPTORS IN DROSOPHILA MELANOGASTER. Yadin Dudai and Shoshana Zvi*, Dept. of Neurobiol., The Weizmann Inst. of Science, Rehovot 76100, Israel.

A particulate fraction prepared from heads of Drosophila melanogaster contains two classes of serotonin-binding sites, as revealed by binding studies performed with [^{3}H]serotonin. A high affinity site has an apparent Kd (at 26° C) of 0.8 nM and a concentration of about 200 fmol per mg protein, and a low affinity site has an apparent Kd of about 100 nM and a concentration of about 1.5 pmol per mg protein. The properties of the high affinity binding sites were investigated. The affinity at 0° C was 0.5 nM, suggesting that the binding does not represent an uptake mechanism. Binding was optimal in the presence of mM concentrations of $MgCl_2$. Low concentrations of non-labeled serotonin, dihydroergotamine, methysergide and metergoline, but not of dopamine and octopamine, displaced [^{3}H]serotonin from its high affinity binding sites. Non-specific binding, i.e., binding observed in the presence of 10^{-5} M serotonin or 10^{-6} M dihydroergotamine, was <10% of total binding at 1 nM [^{3}H]serotonin. Micromolar concentrations of the GTP analoge, Gpp(NH)p, inhibited [^{3}H]serotonin binding. Serotonin has only a marginal stimulatory effect on adenylate cyclase activity in Drosophila head homogenates. Our results indicate that Drosophila head contains high concentrations of serotonin receptors which are clearly distinct from octopamine and dopamine receptors. At least part of the serotonin receptors could be regulated by a guanyl nucleotide. However, the serotonin receptors do not seem to interact in vitro with adenylate cyclase, in contrast, for example, to octopamine receptors in Drosophila. The possibility that rapid changes occur in vitro (e.g., decoupling or desensitization), which render the adenylate cyclase unresponsive to serotonin, should not be excluded.

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282.8 SEROTONIN PRODUCES A SLOW DECREASED CONDUCTANCE EXCITATORY RESPONSE IN INK MOTOR NEURONS OF APLYSIA. John P. Walsh and John H. Byrne, Dept. Physiol. and Cell Biol., Univ. Texas Med. Sch. at Houston, Houston TX 77025

A train of electric shocks to the connectives evokes a long lasting decreased conductance EPSP in the L_{14} ink motoneurons (Carew and Kandel, 1977), which is mediated in part by cell L_{31} (Byrne, 1980). The present study was undertaken to determine the neurotransmitter responsible for the slow EPSP and further examine its cellular mechanisms.

Microejection of serotonin (5-HT) elicited two distinct responses. Placement of the electrode deep beneath the cell generally produced a slow (30-40s) 1 to 3 mV depolarization. The response grew when the membrane potential was artificially depolarized and decreased when hyperpolarized, reversing at about -80mV. In addition the response was associated with a decrease in the input conductance of 8.5 $\pm$ 3.8% (mean $\pm$ S.D., N=13), suggesting that 5-HT acts through a decreased K^+ conductance. In contrast, applying 5-HT to the initial axonal segment generally produced slow depolarizing potentials that increased with depolarization and decreased with hyperpolarization without showing a clear reversal potential. This second type of response was associated with a decrease in input conductance of 2.8 $\pm$ 4.9% (mean $\pm$ S.D., N=17). Replacement of ASW with a Na^+-free solution, however, resulted in a 5-HT response exhibiting kinetics and reversal potential similar to those found with the electrode deep beneath the cell, indicating that this response may be a combination of a decreased conductance to K^+ and increased conductance to Na^+.

Confirming previous studies (Frazier et al., 1967) we found that ACh consistently produced an increased conductance potential which reversed at approximately -55mV. The ACh response had a total duration of only about 15 to 20 sec. The ACh and 5-HT responses were not secondary to the activation of interneurons since comparable responses were obtained in ASW containing 30 mM $CoCl_2$ which blocked synaptic transmission.

To begin to examine the possible role of cAMP in mediating the decreased conductance 5-HT response, 10^{-3}M IBMX was added to the Co^{++}-ASW. The responses resembled 5HT responses in producing a slow depolarization and decreased input conductance. The decreased conductances measured during both the 5-HT and IBMX responses were not due to nonlinear I-V characteristics of the membrane since artificially depolarizing the cell to the same level increased rather than decreased the input conductance. These studies demonstrate that 5-HT may be the transmitter producing the slow EPSP and, furthermore, that the change in K^+ conductance produced by 5-HT may be mediated by cAMP.

282.9 THE IDENTIFICATION AND LOCALIZATION OF A CATECHOLAMINE IN THE MOTOR NEURONS OF A CRUSTACEAN CARDIAC GANGLION. K.A. Ocorr* and A. Berlind. Dept. of Biology, Wesleyan Univ., Middletown, CT 06457.

The cardiac ganglion of the lobster, Homarus americanus, is a relatively simple neuronal oscillator consisting of four small neurons with pacemaker function and five large motor neurons. Electrophysiological analyses have provided extensive information concerning the neurophysiology of the ganglion. Little information exists, however, as to the neurochemistry involved. This investigation provides biochemical and histochemical information as to the neurotransmitter utilized by the cardiac ganglion.

A modification of the glyoxylic acid histofluorescent staining technique was used to stain the ganglion. (Bolstead, G., et al., Comp. Biochem. Physiol., 62C:61, 1978). This treatment results in a distinct fluorescence characteristic of catecholamine containing neurons in the cell bodies and fiber tracts of all five motor neurons. No obvious fluorescence is seen in the small cells. Whole ganglia were screened for their ability to synthesize monoamines and catecholamines from tritiated precursors. Ganglia were incubated in the appropriate precursor and homogenized; the homogenate was separated in one dimension by high voltage electrophoresis (HVE) (Hildebrand, J.,et al., J.Neurobiol., 2:231, 1971). A significant amount of label is incorporated into a compound that comigrates with norepinephrine (NE) (0.539±.02 pmol/ganglion/hr), 60-98% of this label comigrates with NE standard when separated in a second dimension by ascention chromatography. There is no significant incorporation of label into dopamine (DA) (0.027±.01 pmol/ganglion/hr), seratonin (0.024±.01 pmol/ganglion/hr), or octopamine (.0.041±.02 pmol/ganglion/hr with tryamine precursor; 0.42±.01 pmol/ganglion/hr with tyrosine precursor). High pressure liquid chromatography with electrochemical detection (HPLC/EC) was used to determine if endogenous NE is present in the ganglion. An initial separation on an acid washed alumina column was performed to extract the catechols. Subsequent HPLC/EC analysis of the catechol fraction exhibits a peak with the same retention time as that of the NE standard. There is no peak with the same retention time as DA and there is no evidence of the presence of any indolamines.

The data presented demonstrate the ability of neurons in the lobster cardiac ganglion to synthesize and store a catecholamine. The histochemical results suggest that the catecholamine is concentrated in the cell bodies and processes of the five motor neurons only. The HVE and HPLC/EC results are consistent with the identification of NE as the catechol present in these neurons.

282.10 EFFECTS OF VIPOXIN, A 13,000 MW COMPONENT OF RUSSELL'S VIPER VENOM ON RESPONSES TO DOPAMINE AND ACETYLCHOLINE IN APLYSIA. N.T.Slater*,D.O.Carpenter,J.E.Freedman and S.H.Snyder. Center for Laboratories and Research, New York State Dept. of Health,Albany, NY 12201 and Depts of Neuroscience,Pharmacology and Psychiatry, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205.

Previous studies of the effects of Russell's viper venom have demonstrated that this toxin can block K^+-mediated responses to dopamine (DA) and Na^+-mediated responses to acetylcholine (ACh) in Aplysia[1], as well as block the stimulation of cAMP production by DA in rat striatal homogenates[2]. Recently a 13,000 MW protein, vipoxin, has been isolated from Russell's viper venom and shown to interact with α-adrenergic, dopamine and serotonin receptors, but not ACh receptors, in rat brain assayed by a binding technique[3]. In the present study we have examined the effects of vipoxin on the responses of Aplysia neurons to DA and ACh. Neurons were voltage or current clamped with two microelectrodes, agonists were applied by ionophoresis and vipoxin was applied by pressure ejection. ACh-evoked inward currents in abdominal RB and anterior pleural cells were blocked by vipoxin. On many of these cells vipoxin evoked an inward current; cross-desensitization between responses to ACh and vipoxin was observed, and hexamethonium reversibly antagonised both responses. Cl^- and K^+-mediated ACh responses were only slightly affected by vipoxin. Phospholipase A_2 from Russell's viper venom had no effect on responses to ACh. In contrast, inward currents evoked by DA were potentiated in many cases but never antagonised by vipoxin. Both the blockade of ACh inward currents and the potentiation of DA inward currents could be observed on the same anterior pleural cell and these two effects had different time courses. K^+-mediated outward currents evoked by DA in RB and cerebral A cells were not affected by vipoxin. A slow depolarising responses to DA associated with a decrease in membrane conductance could be evoked on small cells in the pleural ganglia. Vipoxin reversibly potentiated this response, while reversibly antagonising excitatory responses of the same cell to ACh. Responses to carbachol were always affected in the same way by vipoxin as responses to ACh. Thus, in contrast to the situation in rat brain[3], vipoxin may have mixed agonist/antagonist effects at excitatory cholinergic receptors in Aplysia. Similarly to its actions at α_1 receptors in vas deferens[3], vipoxin potentiates excitatory responses to DA in this preparation.

[1]Parmentier,J. and Carpenter,D.(1976). Animal, Plant and Microbiol Toxins, 2:179.
[2]Kebabian,J.W.(1978). Brain Res., 144:194.
[3]Freedman,J.E. and Snyder,S.H.(1981). J.Biol.Chem., 256:13172.

282.11 PEPTIDE (PROCTOLIN) INNERVATION OF INSECT SKELETAL AND VISCERAL MUSCLE AND HEART. J.W. Surgeon and M. O'Shea. Dept. Pharmacol. Physiol. Sci., The University of Chicago, Chicago, IL 60637.

Proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) is present in the proctodeum (hindgut) of the cockroach Periplaneta americana where it may be a transmitter. Evidence that it has a widespread CNS distribution among peripherally projecting neurons (see Bishop & O'Shea, J. Comp. Neurol., 1982, in press) suggests a variety of other peripheral targets. To investigate this we have used radioimmunoassay (RIA) and immunohistochemistry to map proctolin and proctolin-containing terminals on skeletal, visceral and cardiac muscle.

In skeletal muscle, whole mount immunohistochemistry reveals proctolin immunoreactive terminations on slow coxal depressor muscles of the hindleg (muscles 177d and 177d') and on muscles of the intersegmental body wall. Some skeletal muscles, for example fast coxal depressor muscles (muscles 178 and 179), apparently are not innervated by immunoreactive terminals. This difference is correlated with proctolin RIA data. Thus, coxal depressor muscles of the 177 group have about 90 fmoles/mg proctolin whereas muscles 178 and 179 contain no detectable levels of proctolin (RIA sensitivity about 4 fmoles/mg). An individual proctolin-containing motoneuron, the slow coxal depressor or Ds neuron (see O'Shea & Bishop, J. Neurosci., 1982, in press), has been identified. This neuron innervates muscles 177, but not 178 or 179. We think, therefore, that the differential distribution of proctolin immunoreactive terminals among these muscles is due to the innervation pattern of the Ds motoneuron.

In visceral muscle, there is widespread proctolin innervation of the gut and ventral diaphragm. The gut immunoreactivity is not confined to the proctodeum, but is also in more anterior parts. The proctodeal immunoreactive terminals are on rectal longitudinal and circular muscles of the gut wall. The proctodeum contains about 300 fmoles/mg proctolin. Part of the innervation of the more anterior parts of the digestive tract appears to derive from immunoreactive cells contained in stomatogastric ganglia.

The heart receives proctolin immunoreactive axons which bifurcate and enter the lateral cardiac nerve from segmental nerves. Although terminations are made in the heart we have not yet determined whether release is directed at cardiac muscle or into the heart lumen for hormonal distribution.

Our observations suggest a widespread neuromuscular transmitter role for a neuropeptide. This is now being investigated by physiological experiments.

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283.1 DECOMPOSITION OF BRAIN TISSUE IN ACID VAPOR: AN IMPROVED METHOD FOR MEASURING METALS IN BRAIN. M.A. Klitenick, W.I. Manton, and C.J. Frederickson. Laboratories for Geochronology and Neurobiology, Univ. of Texas at Dallas, Richardson, Tx. 75080.

Most analytic methods of metal measurement require dissolution of organic matter prior to the assay. Charring (dry ashing) can eliminate organic matter, but charring can also cause loss of analyte by volatilization. Dissolution in warm acid (wet ashing) retains the analyte, but it necessarily contaminates the sample with both the irreducible metal content of the acid(s) and the metal which is leached from the wet ashing vessel. In the present work, we have tested a procedure in which the tissue is decomposed by exposure to the warm vapors of boiling acids. For metal determinations, we used isotope-dilution mass spectrometry.

Lyophilized tissue was placed in a miniature FEP teflon cup (7X9mm) which was placed on an elevated platform inside of a vented teflon bomb. For the first step of decomposition, .5 ml of HNO_3 was placed in the bottom of the bomb, the bomb was closed, and heated just to the boiling point of the acid. After exposure of the tissue to the circulating acid vapor for 24 hrs, the bomb was opened, the HNO_3 evaporated under gentle heat, and the entire process was repeated with .1 ml of $HClO_4$ in the bomb.

Vapor exposure gave complete dissolution of organics: only a white, crystalline, mineral residue was found after the $HClO_4$ was evaporated. Furthermore, two separate tests indicated that none of the metal analyte (zinc) was lost from the sample cup during the procedure. First, when tissue cups were loaded with 10 μg of rare isotope (^{70}Zn) virtually all of the added ^{70}Zn (average 99.1% ± 1.0% S.E.M. for 10 trials) was recovered from the cup after processing, and virtually none (ave .009% ± .0014%) was found elsewhere in the bomb. Second, when individual hippocampi were assayed for zinc using the vapor decomposition, the amounts of endogenous zinc found were no lower than the amounts previously found using conventional wet ashing techniques. Finally, tests for contamination (done by processing empty tissue cups) showed that the average "blank" contained only 9.2 ng (± 1.3 for 10 trials) of extraneous zinc; with wet ashing, our prior contamination level had averaged 43 ng.

An average contamination error of 9.2 ng permits samples containing 200 ng of zinc to be assayed with 5% accuracy. In tests with 1/4 sections of rat hippocampus (each containing about 200 ng of zinc) we have indeed found a consistent pattern, with zinc levels highest in the septal pole and declining gradually through the temporal pole. Septal to temporal zinc concentrations for a single, representative hippocampus were 83, 78, 77, and 71 PPM, respectively. The results indicate that vapor dissolution is a clean and reliable way to prepare tissue for metal assays.

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283.2 SELECTIVE UPTAKE OF ZINC IN SUB-REGIONS OF HIPPOCAMPAL SLICES IN VITRO. Gailyn A. Howell and Christopher J. Frederickson. Laboratory for Neurobiology, University of Texas at Dallas, Richardson, Tx. 75080.

The zinc associated with the hippocampal mossy fibers has been studied extensively by histoanalytic methods, but relatively little is known about the utilization or metabolism of that zinc. As a prelude to investigations of relationships between impulse activity and uptake/release of zinc in the mossy-fiber region, we have examined the kinetics of high-affinity zinc uptake in selected brain regions and have done pilot experiments on the effects of electrical stimulation on zinc uptake in hippocampus.

Slices of mouse hemicerebrum (450 μm) were conventionally prepared and preincubated for 1 hr in Yamamoto's medium prior to incubation in media with ^{65}Zn. Following incubation, the slices were rinsed, dissected, dried, and assayed by gamma counting.

Zinc uptake into hippocampal slices increased linearly with concentration up to 100 μM (and with time up to 360 min.), but showed saturation at higher zinc concentrations and longer incubation times. Lineweaver-Burke analysis ($r > .99$) gave a Vmax of 9.2 ng/mg/hr and Km of 17.7 μM. Neocortical slices showed uptake similar to whole hippocampus ($r > .98$, Vmax = 10.1 ng/mg/hr, Km = 16.6 μM), but slices of striatum exhibited a higher Km ($r > .98$, Vmax = 9.6 ng/mg/hr, Km = 25 μM).

Zinc uptake in hippocampal and neocortical slices was suppressed (42-50%) by lowered incubation temperature (4° C), and was suppressed (41-54%) by ouabain (100 μM) added to the medium.

Whereas whole hippocampal slices did not differ appreciably from cortex in zinc uptake, regional differences within the hippocampus were marked. When hippocampal slices were incubated in 20 μM zinc (30 min), then lyophilized and dissected into regions, the amounts of incorporated zinc found in the hilus (5.7 ng/mg), the dentate gyrus (6.0 ng/mg), and CA3 (5.0 ng/mg) were up to 60% greater than that found in CA1 (3.5 ng/mg).

Preliminary data indicate that electrical stimulation increases zinc uptake in all 4 hippocampal regions. Pulses (3.5 μA/100 um^2; 0.4 msec; 25 pps; 25 min) delivered via a 2mm platinum wire encircling the dentate gyrus increased zinc uptake (20 μM medium concentration) by 29% to 45% in all 4 regions; slower stimulation (12.5 pps) yielded a smaller, but consistent increase.

The present finding of elevated zinc uptake in regions innervated by mossy fibers (hilus and CA3) and in the vicinity of the granule cells (dentate gyrus) suggest that the granule cell/mossy fiber system accumulates zinc by mechanism(s) distinct from those of other regions. Moreover, the enhancement of zinc uptake by electrical stimulation favors the notion that zinc utilization is partly linked to electrophysiological activity in the tissue.

283.3 QUANTITATIVE AUTORADIOGRAPHY OF LOCAL BRAIN PROTEIN SYNTHESIS. VALIDATION FOR TRITIATED VALINE. B.E. Dwyer*, P. Donatoni* and C.G. Wasterlain* (SPON: R.Nishimura). Epilepsy Research Laboratory, V.A.M.C. Sepulveda, CA 91343 and the Department of Neurology and Brain Research Institute, UCLA, Los Angeles, CA.

Recently we have described a quantitative autoradiographic method for measuring local rates of brain protein synthesis (LBPS) using (1-14C) L-valine (Dwyer et al., Soc. Neuroscience 7:789,1981; Dwyer et al. Neurochem. Res. 1982, in press). We have now validated this method for use with tritiated L-valine. Four-day old rats maintained at 37°C were injected with 150mM L-(3,4-3H)valine (10 μmoles/g, 0.2 μCi/umole, IP) and were sacrificed at 30, 60, and 120 min when brains were used to generate the standard curve or at 90 min when brain was taken for measurement of LBPS. For generating the standard curve 1/2 forebrain from each rat was acid precipitated and delipidated and valine incorporation into protein was measured chemically. The injection of 150mM valine results in a constant specific activity of brain acid-soluble valine for at least 2 h.(mean value = 82.3% of injectate). Over the same period incorporation of (3H) into protein was linear. The other 1/2 forebrain from each of the animals used for the standard curve was homogenized (undiluted), frozen and cut into 20 micron sections. Frozen sections of homogenate were sequentially acid washed (10% TCA containing 0.1% valine) and water washed and exposed to LKB ultrofilm 3H in standard X-ray cassettes. A linear relationship was found between the optical density of film exposed to homogenate sections and the amount of 3H incorporated into protein thus validating the use of this method for quantitative measurement of LBPS by autoradiography. Brains taken for measurement of LBPS were frozen, cut in 20 micron coronal sections acid washed and exposed to film as described above.The rate of protein synthesis measured in cerebral cortex using this method was 2.82±0.19 % per hour (mean ± S.D.) (N=4) which is in excellent agreement with that measured previously using (1-14C) valine (2.72±0.12 % per hour, N=4). There are several advantages to this method. It is ideally suited for young or small animals where arterial and venous catheterization may be impossible or impractical or where surgery and immobilization as required in the method described by Smith et al. (Trans. Amer. Soc. Neurochem. 11:94, 1980) are undesireable. Since flooding amounts of amino acid are injected the problem of estimating precursor pool specific activity particularly under pathological conditions is minimized. Furthermore, 3H amino acids are less expensive than (1-14C) labelled amino acids and 3H is amenable to the use of sensitive liquid emulsions and stripping films (eg. Kodak AR-10) so that a quantitative estimate of protein synthesis can be obtained at the cell level. Supported by grant #13515 from NINCDS and by the research service of the Veterans Administration.

283.4 BIPHASIC ALTERATIONS IN ATPase ACTIVITIES FOLLOWING CEREBRAL ISCHEMIA AND RECIRCULATION. W.J. Goldberg*, R. Busto*, H. Kurchner* and M.D. Ginsberg* (SPON: P. Scheinberg). Cerebral Vascular Disease Research Center, Dept. Neurol. Univ. Miami Sch. Med., Miami, FL 33101.

ATPase activities were measured colorimetrically in 3 - 6 mg samples (Kogure et al., Brain Res., 195, 95-109, 1980) taken from medial cortex (MED), dorsolateral cortex (DOR), lateral cortex (LAT), hippocampus (HIPP), thalamus (THAL) and striatum (STR); left and right side samples were taken from each brain. Control levels, expressed as nmol Pi produced/minute/mg protein, were: MED, 292.3; DOR, 320.1; LAT, 302.7; HIPP, 241.7; THAL, 438.2 and STR, 270.5. Ischemia was induced in anesthetized and paralyzed male Wistar rats using the model of Pulsinelli and Brierley (Stroke,10, 267-272, 1979). Following 30 min. ischemia, a 30-40% increase ($p < 0.01$) in (Na+, K+)-ATPase activity above control level was observed in DOR, LAT, HIPP and THAL. ATPase activity returned to within 10% of control levels after 1 hr. recirculation. At the end of 4 hrs. recirculation (Na+, K+)-ATPase activities fell to 30-35% below control levels ($p < 0.5$); in THAL, no decrease in enzyme activity was observed. In MED, (Na+, K+)-ATPase activity was elevated 20% (NS) at the end of the ischemia but continued to rise to 50% above control ($p < 0.1$) by the end of 1 hr. recirculation. ATPase activity fell to 35% below control levels ($p < 0.5$) after 4 hrs. recirculation. Alterations in ATPase activity in STR were similar in time course to those observed in MED but were of non-significant magnitudes.

Biphasic alterations in ATPase activity due to detergent action have been reported by Stahl (Arch. Biochem. Biophys., 154, 56-67, 1973). During ischemia many lipid breakdown products having detergent-like actions (free fatty acids, diacylglycerols and lysophospholipids) are formed. The observed alterations in ATPase activities may be related sequential alterations in membrane structure.

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283.5 Mechanisms of Irreversible Anoxic Damage in the Rat Hippocampal Slice. Ira S. Kass and Peter Lipton. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

We are using the hippocampal slice to study irreversible brain damage. The perforant path is stimulated once every 15 seconds and the evoked population spike is recorded in the dentate granule cell layer; its magnitude is measured. We have previously demonstrated that the extent of the fall in ATP during 10 minutes of anoxia correlates with the extent of irreversible loss of the evoked response. Thus, when ATP levels during anoxia were maintained by preincubating the slices with creatine, the extent of irreversible damage during anoxia was markedly reduced.

Ten minutes of exposure to anoxia leads to almost complete irreversible loss of the evoked response one hour after returning to normoxic buffer. There is an associated reduction of ATP from 13.9 to 9.7 nm/mg protein. When sub-maximal doses of sodium azide are used to directly reduce ATP to the level found one hour after anoxia the evoked response is diminished by only 45%. Thus the reduced postanoxic ATP levels are not sufficient to explain the loss of the evoked response.

Intracellular K in the hippocampal slice is reduced by 27% one hour after anoxia and this is associated with almost complete loss of the response. 10^{-6}M ouabain added to the normoxic slices reduces K^+ by 50% but only partially blocks transmission. Thus, a membrane depolarization as a result of pump inhibition cannot explain the irreversible loss of transmission. Therefore, neither the postanoxic ATP nor the postanoxic K changes appear to be the cause of irrerversible signal loss.

We next examined events during the anoxic period which could be leading to the irrerversible damage. In addition to a large reduction in ATP, from 13 to 4 nm/mg protein, there is a large increase in intracellular Na (from 73 to 132 mM). Both these changes would tend to increase cytosolic Ca^{2+}. This tendency would be much exaggerated by the inhibition of electrontransport in the mitochondria. We tested the hypothesis that increased cytosolic Ca^{2+} might be leading to the irreversible functional loss. We did this by removing extracellular Ca^{2+} during the 10 minute anoxic period. In this case the evoked response recovers to 66% of its original value, one hour after the anoxic exposure. There is no significant recovery in normal buffer. This result suggests that the influx of Ca during anoxia is a major factor leading to irrerversible transmission loss.

We are currently trying to differentiate between three different sites of Ca action:

a) the reduction of cell ATP
b) the activation of a phospholipase
c) the activation of a protease.

283.6 EFFECTS OF GLUCOSE AND OTHER CARBOHYDRATES ON CEREBRAL ISCHEMIA IN THE MOUSE. F. A. Welsh, R. Sims* and A. McKee*. Division of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104.

Pretreatment of experimental animals with glucose greatly exacerbates brain damage caused by cerebral ischemia (Myers, R.E., Neurology, 26:345, 1976). In order to study the mechanism of the effect of glucose, we administered several carbohydrates, structurally similar to glucose, 30 min prior to cerebral ischemia in the mouse. Adult male mice, 20-25 grams, were anesthetized with tribromoethanol. The common carotid artery on the right side was occluded and the animal was placed in a chamber containing 10% oxygen and 90% nitrogen. Following 30 min of hypoxia, the animal was returned to room air, and the carotid occluder was removed. After 60 min of normothermic recovery, the mouse was frozen by immersion in liquid nitrogen. The frozen brain was sampled regionally for assay of ATP.

In animals administered glucose (15 mmol/kg body weight, I.P.), ATP levels at 60 min of recovery were only 0.27 ± 0.14 mmol/kg (Mean ± SEM, N = 7) in the cerebral cortex ipsilateral to the previously occluded carotid artery. In the contralateral cortex, ATP levels were 2.52 ± 0.13 mmol/kg, a value not significantly different from unanesthetized, normoxic mice (2.71 ± 0.05 mmol/kg, N = 10). By contrast, in animals pretreated with saline, ATP levels in the ipsilateral cortex recovered to 1.84 ± 0.17 mmol/kg, N = 8. Animals frozen at 30 min of hypoxia-ischemia without recovery had low levels of ATP in the ipsilateral cortex (0.30 mmol/kg) in both saline and glucose pretreated groups. However, end-insult levels of lactate were significantly higher in the animals pretreated with glucose (lactate = 39.2 ± 1.5 mmol/kg, N = 10) than those pretreated with saline (lactate = 30.1 ± 2.5 mmol/kg, N = 9).

Pretreatment with mannose, but not galactose nor 3-O-methylglucose, was able to mimick the deleterious effect of glucose on posthypoxic recovery of ATP. Thus, in animals pretreated with 15 mmol/kg mannose, ATP levels in the ipsilateral cortex were only 0.15 ± 0.10 mmol/kg, N = 4, compared to 1.96 ± 0.27, N = 4 (galactose pretreatment) and 1.54 ± 0.14 mmol/kg, N = 4 (3-O-methylglucose pretreatment).

These results demonstrate that carbohydrates which are not metabolized by brain (galactose and 3-O-methylglucose) do not exacerbate the effects of hypoxia-ischemia. Carbohydrates which are metabolized by brain (glucose and mannose) may be harmful because of the greater build-up of lactic acid during the period of hypoxia-ischemia.

283.7 INHIBITION OF OXIDO-REDUCTION IN RESPIRATORY CHAIN NICOTINAMIDE ADENINE DINUCLEOTIDE BY CARBON DIOXIDE. Carlos Rodríguez-Estrada. Cátedra de Fisiología, I.M.E. Facultad de Medicina, U.C.V., Caracas, Venezuela.

In an earlier report (Rodríguez-Estrada, Carlos Neuroscience Abs. 3:321, 1977) was shown that carbon dioxide blocked the metabolic response after a short period of peripheral nerve stimulation. Also the increase of carbon dioxide partial pressure changes the level of NADH in the soma of dorsal root ganglion neuron (Rodríguez-Estrada, Carlos Neuroscience Abs. 5:91, 1979) towards a new level of decreased NADH.

In this work it was expected that the change of hydrogen ion concentration brought about by increasing carbon dioxide partial pressure inhibits the respiratory chain.

Fluorometric determinations of NADH were done on in vitro preparations of frog dorsal root ganglion (Rana palmipes spix) as previously reported, pH and pO_2 were measured simultaneously. In one group the preparation was placed in O_2 atmosphere and the O_2 was replaced with N_2, measuring the oxidation during N_2 replacement. Later, in the same preparation O_2 was replaced with CO_2-O_2 gas mixture and this was sustituted with CO_2-N_2 and measuring the oxidation during CO_2-N_2 replacement with CO_2-O_2 mixture.

Changing O_2 for N_2, and after 3-4 minutes in N_2 atmosphere, an increase of NADH was observed and a full reduction was achieved in one or two minutes later. When CO_2 2.5%-O_2 97.5% gas mixture was replaced with CO_2 2.5%-N_2 97.5% the NADH level increased after 5-6 minutes as expected in an O_2 free atmosphere. And in a preparation with CO_2 5%-O_2 95% gas mixture was replaced with CO_2 5%-N_2 95% gas mixture the level did not change after 7 minutes in this O_2 free atmosphere. The reduced level reached in CO_2 2.5%-N_2 97.5% was the same or smaller than that observed in N_2, which varied from 8 to 18%. Removing the CO_2 5%-O_2 95% gas mixture with O_2 and later replacing O_2 for N_2 the level of NADH increased as expected and described above.

These results indicated that oxido-reduction of the respiratory chain was blocked with a critical increase of carbon dioxide and supports the idea of a change of the equilibrium constant of NAD/NADH due to an increase of hydrogen ion concentration.

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283.8 COMPARISON OF LIPIDS AND LIPID METABOLISM IN A HUMAN GLIOMA CELL LINE WITH OLIGODENDROGLIA. S.E. Poduslo, K. Miller* and Y. Jang* Dept. of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

The permanent human glioma cell line D-54 MG was established from a glioblastoma (maintained at Duke University in D. Bigner's laboratory). In culture the cells have a polygonal shape, dense cytoplasm, and numerous intertwining processes. The cell line and one of its single cell-derived clones exhibit some properties of oligodendroglia. These include high levels of 2',3'-cyclic nucleotide 3'-phosphodiesterase enzyme activity and a pronounced response to dibutyryl cyclic adenosine monophosphate (further differentiation). Markers for astrocytes (S-100 protein and glial fibrillary acidic protein) are absent. Interestingly, the cells react with a polyclonal antiserum specific for oligodendroglial surface components.

The glioma cells were further examined for glycolipids characteristic of oligodendroglia. The glioma cells have only 2% of the total lipids as galactolipids whereas oligodendroglia have about 10%. The ratio of cerebrosides to sulfatides was 1:1 in glioma cells while it was almost 5:1 in oligodendroglia. Both glycolipids from the glioma cells showed altered mobilities on thin layer chromatograms, suggesting possible changes in the fatty acids. The most striking changes in phospholipids were the increases in sphingomyelin and phosphatidylinositol and the decrease in phosphatidylethanolamine for the tumor cells when compared to oligodendroglia.

When incorporation of various radiolabeled substrates into specific lipids was examined, it was found that 14% of [^{3}H] galactose was incorporated into cerebrosides in the glioma cells while 56% was incorporated into cerebrosides by oligodendroglia. The mitotic properties of the tumor cells are reflected in the high levels of incorporation of [^{3}H]choline, [^{3}H]glycerol, and [^{3}H]acetic acid into various parts of the molecule of phosphatidylcholine, indicating the increased amounts of plasma membranes that the cells generate. An interesting finding was the relatively high level of incorporation into phosphatidylinositol in the glioma cells. In addition, the ganglioside patterns were less complex for the glioma cells. Thus the glioma cells have greatly decreased amounts of glycolipids when compared to oligodendroglia. This finding is consistent with the theory that the loss of glycolipids on the cell surface may lead to the loss of regulation of contact inhibition.

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283.9 EVIDENCE FOR CARNITINE OCTANOYLTRANSFERASE IN ISOLATED SKELETAL MUSCLE MITOCHONDRIA. C.F. Ansevin and C.L. Hoppel*. Department of Neurology, Case Western Reserve University and Research Service, Veterans Administration Medical Center, Cleveland, Ohio 44106.

Skeletal muscle mitochondria were isolated from the hindlimbs of male Sprague-Dawley rats. These mitochondria oxidized glutamate; pyruvate and malate; alpha-ketoglutarate; succinate (in the presence of rotenone); palmitoylcarnitine with malate; palmitoyl CoA with malate and l-carnitine; octanoylcarnitine with malate; octanoyl CoA with l-carnitine and malate; and l-acetylcarnitine with malate. Octanoate was not oxidized probably owing to inactivation of octanoyl CoA synthetase by the proteolytic enzyme Nagarse during the isolation procedure.

The mitochondrial inner membrane is impermeable to CoA derivatives of fatty acids. However, the acylcarnitine derivatives of fatty acids are permeable to the mitochondrial inner membrane. Octanoyl CoA was not oxidized until l-carnitine was added to the incubation media. The carnitine-dependent rates of mitochondrial oxidation of octanoyl CoA were slower than those of octanoylcarnitine. ADP-stimulated (State 3) rates of oxidation of octanoylcarnitine (with malate) were 177 nanogram atoms (ngA) Oxygen/min./mg. protein ± S.D. of 13. Palmitoylcarnitine oxidation rates (206 ngA O/min./mg. protein) were very similar to those of palmitoyl CoA with l-carnitine (213 ngA/min./mg. protein with a S.D. of 13).

This suggests that there may be a carnitine octanoyltransferase (COT) A and B [analogous to carnitine palmitoyltransferase (CPT) A and B] in rat skeletal muscle mitochondria catalyzing the reactions: octanoyl CoA + l-carnitine → octanoylcarnitine + CoA
octanoylcarnitine + CoA → octanoyl CoA + l-carnitine
and in the process allowing octanoyl CoA to enter the matrix for beta-oxidation. Whether the COT activities are due to different substrate specificities of CPT A and B or owing to separate enzyme activities is not known and further studies are planned.

283.10 ADENOSINE MAY CAUSE EARLY INHIBITION OF SYNAPTIC TRANSMISSION DURING ANOXIA. Peter Lipton and Karen Robacker*. Dept. of Physiology, Univ. of Wisconsin, Medical School, Madison, WI 53706.

The basis for the rapid decay of synaptic transmission in brain during anoxia is not known. Depolarization is probably an important factor (Lipton and Whittingham, J. Physiol. 287, 427) but, other factors may well be involved. Metabolically there is a correlation between the rate of fall in ATP concentration and the rate of decay of transmission (Lipton and Whittingham, J. Physiol. 325, 51). One result of the decline in tissue ATP during anoxia is a release of adenosine into the medium (Fredholm and Vernet, Acta Physiol Scand. 106: 97). Adenosine has been shown to be a potent inhibitor of synaptic transmission at many cerebral synapses, including those in the hippocampus (Dunwiddiee and Hoffer, Br. J. Pharmacol. 69: 59). Thus, the release of adenosine may inhibit synaptic transmission early during anoxia. We have begun to test this hypothesis using the hippocampal slice preparation.

Guinea pig hippocampal slices are submerged in a chamber in standard Krebs-bicarbonate buffer at 35°C. The perforant path is stimulated and the population spike is measured in the dentate granule cell layer. Anoxia is introduced by flowing buffer equilibrated with 95% N_2-5% CO_2 into the chamber.

Both 3-isobutyl methyl xanthine (IBMX) and adenosine deaminase (ADA) block the inhibitory effect of exogenous adenosine in the hippocampal slice. If adenosine is blocking transmission during *anoxia, then IBMX and ADA should slow down the rate at which the* block occurs. In standard buffer the population spike begins to fall at one minute after the onset of anoxia; it becomes 1/2-maximal at 2.7 minutes. 0.4 mM IBMX rapidly increases the population spike in normoxic buffer by 25%. The spike begins to fall at 2.5 minutes after the onset of anoxia and it becomes 1/2-maximal at 4.4 minutes. Thus, IBMX produces about a 90 second delay in the onset of the fall in transmission. ADA (2 U/ml) had a smaller effect on the size of the population spike in normal buffer and a smaller, but significant, effect on the rate of decay of the response during anoxia: the fall was delayed by 45 seconds rather than 90 seconds.

We determined whether IBMX was exerting its protective effect by maintaining tissue ATP levels during anoxia. In fact, IBMX exaggerated the fall in ATP. In control tissue ATP fell from 15 to 14 nM/mg P, while with IBMX, ATP fell to 11 nM/mg P ($P < .01$ for difference) after 3 min of anoxia.

Thus, the data suggest that release of tissue adenosine contributes to early inhibition of transmission during anoxia.

283.11 MICROFIBER, DUAL WAVELENGTH, REFLECTION SPECTROPHOTOMETRY OF CYTOCHROME OXIDASE FROM SMALL, SUBSURFACE AREAS OF BRAIN IN SITU, R.L. Novack*, T.J. Sick* and M. Rosenthal. Dept of Neurology, University of Miami School of Medicine, Miami, FL 33101.

Dual wavelength spectrophotometry of reduction/oxidation shifts of mitochondrial respiratory chain components has been a useful tool to study oxidative metabolic activities of mitochondria and of brain and other tissues in vitro. By monitoring reflected light at 605 nm (the wavelength of maximal absorption of reduced cytochrome c oxidase), it has become possible to follow redox reactions of this cytochrome from intact tissues such as brain. However, earlier instruments were limited to recordings from tissue surfaces. We report here that fiber optic technology now allows such measurements to be made from microareas within brain tissues. Studies were performed on rats anesthetized with sodium pentobarbital and artifically ventilated. Single glass fibers (20u in diameter) were interfaced with a photomultiplier tube to collect reflected light after its delivery to the tissue through large diameter light guides. Cytochrome oxidase redox status was derived by subtracting reflected light at 605 nm from that at 590 nm to compensate for changes in scattering, blood volume and oxygenation. These studies demonstrate that light at these wavelengths will penetrate from the cortical surface through the entire gray mantle in the rat. Light penetration falls off markedly at the level of the underlying white matter. Increasing or decreasing the fraction of inspired oxygen (FiO_2) produced oxidative or reductive shifts (respectively). Only very small reductive responses to hypoxia were recorded in white matter. When microfiber probes were placed upon subpial blood vessels, changes in FiO_2 produced expected changes in blood volume but redox shifts of cytochrome oxidase were not recorded. Such results add additional confirmation to the accuracy of reference wavelength compensation for vascular changes. Electrical stimulation of the cerebral surface produced local oxidative shifts of cytochrome oxidase which decreased in amplitude with increasing lateral distance from the stimulus. Amplitudes of these responses appears to be a complex function of the depth of penetration of the microfiber as proximity to the layers of greatest mitochondrial density increases but electrical effects of the surface stimulation decrease. When spreading depression was produced, negative shifts of the cortical extracellular voltage and oxidative responses of cytochrome oxidase were recorded. Such oxidative responses were faster and recovered sooner than those proviously recorded from larger areas of the cerebral surface and were more coincident with the voltage shifts. These data demonstrate the feasibility of the microfiber procedure in the study of oxidative metabolic activity of the brain.

283.12 A NEW METHOD FOR RAPID FREEZING OF CAT BRAIN. M.S. Yang*, D.P. Becker* and R.L. Hayes. (SPON:Anne Snow). Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298

This study introduces a new method for rapid freezing of cat brain. Temperature changes in two brain sites and metabolite levels in 5 brain regions were compared with the freezing method of Welsh et.al. (J. Neurochem. 31:299, 1978)

With the Welsh method, a hollow styrofoam cup was placed on top of the skull into which liquid nitrogen was poured. The new method employed a styrofoam box with a removable cover. The cover was cut into two half. The front piece was inserted into the mouth and the back piece was fitted around the neck. The bottom of the box was cut away and the remaining body was placed on top of the cover. Liquid nitrogen was poured into the inverted box which now contains the upper part of the head. Temperature was measured by chromel-alumel probe implanted either at the base of the brain resting against the ventral hypothalamus, or in the forth ventricle lodging between the cerebellum and the medulla. Glucose, ATP,P-Creatine and lactate were measured in samples from cortex, thalamus, hypothalamus or pons, cerebellum and medulla according to the methods of Lowry and Passonneau (A Flexible System of Enzymatic Analysis, 1972).

When the Welsh method was used, more than 15 min was required to bring the base of the brain to 0°C. The freezing front did not reach the hypothalamus until 6 minutes after liquid nitrogen was applied and temperature fell linearly during the next 20 min at a rate of 3°C/min. At the medulla, temperature fell to 0°C at 8 min after the onset of freezing. With the styrofoam box method, the temperature of the hypothalamus began to drop at 2 min and decreased at a rate of 30°C/min thereafter, bringing the temperature to 0°C at 4 min. A similar freezing rate was also found in the medulla although the freezing front arrived slowly during the first 3 min. Freezing time to 0°C for the medulla was 5 min.

The levels of metabolites in deep brain regions frozen either by the cup or the box method were the same. Lactate levels of any of the 5 brain regions studied were not elevated indicating no extensive ischemia in brains frozen by either method.However, the use of styrofoam box provides shorter freezing time allowing improved temporal resolution for studying dynamic metabolic changes frequently observed in pathological condition. Supported by NIH Grant #NS-12587

283.13 *OXIDATIVE ENZYME ACTIVITY OF SLOW-TWITCH OXIDATIVE AND FAST-TWITCH GLYCOLYTIC* α-MOTONEURONS. D. W. Sickles* and T. G. Oblak*. (SPON: G. Doetsch). Dept. of Anatomy, Medical College of Georgia, Augusta, GA 30912.

Metabolic heterogeneity among mammalian muscle fibers is well documented. The relative dependence of muscle fibers on oxidative metabolism determines their resistance to fatigue (Burke and Tsairis, Ann NY Acad Sci 228:145, 1974). Variation in the activities of NADH-diaphorase, acid phosphatase and α-glucan phosphorylase among rat lumbosacral α-motoneurons have been observed (Sickles DW, McLendon RE. Submitted Exp Neurol). The present study was designed to determine whether metabolic variation in α-motoneurons is related to the type of muscle fibers innervated. Under pentobarbital anesthesia, the rat left soleus (84% slow-twitch oxidative) and right tensor fascia lata (94% fast-twitch glycolytic) muscles were injected with 3.5 and 10.5 µl of 25% horseradish peroxidase, respectively. Sixteen to twenty hours later the animals were decapitated, the spinal cord removed (de Sousa and Horrocks, Develop Neurosci 2:115, 1979) and 5 µm serials sections were cut on a Bright cryostat. Alternate serial sections were processed for demonstration of NADH-diaphorase and retrogradely transported HRP. The motoneurons innervating the soleus and tensor fascia lata muscles were identified on the slides processed for peroxidase activity; the relative NADH-diaphorase activity of these identified motoneurons was determined on a Zeiss Zonax scanning and integrating microdensitometer. The optical density (O.D.), directly proportional to enzyme activity, was determined at ten spots (5 µm diameter) in each cell and averaged. Histograms plotting the number of cells with a given O.D. demonstrated a bimodal distribution with insignificant overlap (5-12% overlap). The overlap may correspond to the presence of motoneurons innervating the fast-twitch oxidative-glycolytic fibers found in both muscles (6-16%). The enzyme activity was greater in motoneurons innervating the SO fibers of the soleus than the FG fibers of the tensor fascia lata. The data indicates that motoneurons may have varying resistance to fatigue which is matched to that of the muscle fibers. The varying dependence of motoneurons on oxidative metabolism may result in a selective susceptibility to pathogenic agents.

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283.14 DIFFERENTIAL LABELING OF SLOW-TWITCH OXIDATIVE AND FAST-TWITCH GLYCOLYTIC α-MOTONEURONS WITH HORSERADISH PEROXIDASE. T. G. Oblak* and D. W. Sickles*. (SPON: M. J. Mulroy). Dept. of Anatomy, Medical College of Georgia, Augusta, GA 30912.

Qualitative and quantitative differences in the staining of HRP back-filled α- and γ-motoneurons have been previously observed (Strick et al., Brain Res 113:582, 1976). A more recent report (Neuhuber and Niederle, Neurosci Lett 20:131, 1980) has shown a time differential in labeling of small vs large sensory cells and suggested a similar difference in different-sizes of motoneurons. With an extremely sensitive HRP technique developed for use on 5 µm thick sections (Sickles and Oblak, submitted Neurosci Meth), we have observed a distinct difference in staining of HRP-backfilled α-motoneurons innervating the slow-twitch oxidative (SO) muscle fibers of the rat soleus from the neurons innervating the fast-twitch glycolytic (FG) fibers of the tensor fascia lata (TFL) muscle. We have further investigated this observation to determine the reason for the quantitative difference in exogenous protein transport in α-motoneurons. The volume of HRP injected into the muscle was adjusted to compensate for wet weights of the Sol and TFL muscles, without any effect on staining. Animals were injected at 4, 6, 8, 10, 12, 14, 16 and 26 hours prior to sacrifice to determine whether the transport rate was different. The time of appearance of HRP within the TFL and Sol motoneurons was 8 and 12 hours, respectively. The length of nerves from muscle to spinal cord was 11.9 cm for soleus and 6.85 cm for TFL. The rate of transport was not significantly different; 1 cm/hour for soleus, 1.17 cm/hour for TFL. The average innervation ratio of the muscles was found to be 1:66 (29:1909) for the TFL and 1:71 (35:2493) for the soleus. Average size of the motoneurons varied by 70%. Therefore, the lower HRP staining in TFL as compared to Sol motoneurons may be accounted for, at least in part, to a dilution of the same amount of HRP within a larger cell. Quantitation of HRP within the cell is currently being determined to establish whether it is inversely proportional to cell size. Two other factors, activity of the motor unit and lysosomal enzyme activity (acid phosphatase), are also being examined to establish their role in determining the quantity of exogenous protein within motoneuron cytoplasm following intramuscular injections.

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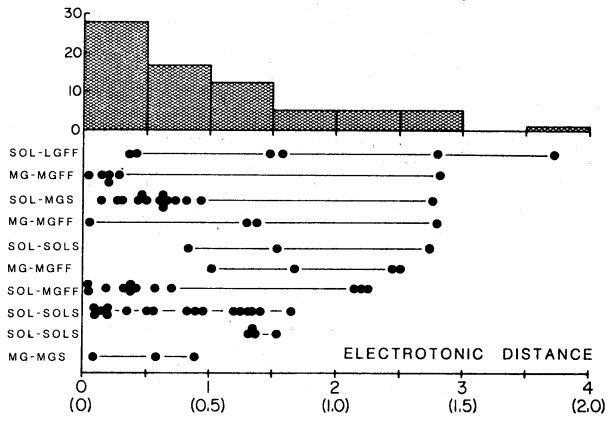
284.1 ESTIMATES OF ELECTROTONIC DISTANCE OF GROUP Ia CONTACTS ON CAT α-MOTONEURONS: AN HRP MORPHOLOGICAL STUDY. L.L. Glenn, R.E. Burke, J.W. Fleshman and A. Lev-Tov*. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205

The anatomy of presumed synaptic contacts made by HRP-labeled group Ia arborizations on functionally-identified, HRP-labeled α-motoneurons has been reported previously (Brain Res. 160:347, 1979; Soc. Neurosci. Abstr. 6:713, 1980). We used such material to estimate the electrotonic distance (X_j) between individual Ia contacts and motoneuron somas from measurements of lengths and diameters of dendritic paths, making different assumptions for specific membrane (R_m) and cytoplasmic (R_i) resistivities. In 3 spinal cords, detailed reconstructions were made of 10 contact systems (an afferent-motoneuron pair; 10 triceps surae α-motoneurons [5 type FF and 5 type S]; 4 group Ia afferents; 7/10 homonymous connections). The dot displays below show estimates of X_j for each contact system; the histogram summarizes the distances of all 73 individual contacts. The abscissa scale from 0 to 4 resulted from using R_m = 2000 ohm-cm^2; the scale from 0 to 2 (parentheses) used R_m = 8000 ohm-cm^2 (R_i = 70 ohm-cm). Of the 10 contact systems studied, 8 showed contacts spread over X_j>1 (0.5 with the higher R_m). Mean X_j (N=73) was 0.97 ± 0.85 (S.D.; 2000 ohm-cm^2) or 0.49 ± 0.82 (S.D.; 8000 ohm-cm^2). Fewer of these contact systems terminated within restricted electrotonic loci than might have been expected on the basis of previous physiological evidence.



284.2 THE ABSENCE OF SPECIFIC DYE COUPLING AMONG FROG SPINAL MOTONEURONS. Sue Powell and Monte Westerfield, Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

A double labeling technique was developed to examine the specificity of dye coupling among frog spinal cord motoneurons. A pool of electrically coupled motoneurons was retrogradely prelabeled with rhodomine-conjugated HRP (R-HRP), a dye molecule that is too large to pass between coupled cells. Then single motoneurons inside and outside this pool were injected with lucifer yellow (LY), a smaller molecule that passes between coupled neurons. The pattern of subsequent labeling was examined to determine if LY spreads specifically among cells that are electrically coupled.

Previous work has shown that motoneurons innervating the medial head of the triceps muscle in the front limb of the frog are electrically coupled to each other but not to motoneurons innervating other muscles. Similarly, motoneurons innervating the internal and external heads are coupled only within their pool. Application of R-HRP to the cut end of one of these nerves prelabeled the motoneurons that innervated one muscle and thus identified a pool of electrically coupled motoneurons. Subsequent intracellular recording and antidromic stimulation identified single motoneurons either within or outside the prelabeled pool. Pressure and iontophoresis were then used to inject LY into these identified motoneurons.

In 7 animals (13 injections) only a single motoneuron was labeled by each intracellular LY injection. No dye coupling was observed. In 2 animals (4 injections) injection of single motoneurons resulted in dye spread and the labeling of many cells. The secondarily labeled cells were found to lie randomly within and outside the pool of electrically coupled cells, identified by R-HRP. Most often, secondarily labeled cells were found near the electrode track.

We conclude that frog spinal cord motoneurons are not dye coupled under normal physiological conditions and that secondary labeling, when it occurs, is due to leakage and non-specific uptake either by damaged or neighboring cells.

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284.3 A QUANTITATIVE ANALYSIS OF THE UNUSUALLY LARGE DENDRITIC TREES OF MOTONEURONS IN THE UPPER CERVICAL SPINAL CORD OF THE CAT. P. K. Rose, S. A. Keirstead* and S. J. Vanner*. (SPON: J. V. Milligan) Dept. of Physiology, Queen's University, Kingston, Ontario Canada K7L 3N6.

Two recent studies (Ulfhake and Kellerth, J. Comp. Neurol. 202: 571, 1981; Egger and Egger, Neurosci. Abstr. 7: 419, 1981) using intracellular injections of horseradish peroxidase (HRP), have provided detailed quantitative measurements of the dendritic trees of motoneurons innervating hindlimb muscles. Comparisons of the data reported in these studies with measurements of total dendritic length of dorsal neck muscles (Rose, J. Comp. Neurol. 197: 395, 1981) suggest that motoneurons in the upper cervical spinal cord may have substantially larger dendritic trees than those in the lumbosacral spinal cord. The purpose of the present investigation was to examine these quantitative differences in greater detail.

Motoneurons were intracellularly stained with injections of HRP and their dendritic trees were reconstructed from serial histological sections. The subsequent quantitative analysis took into account the tortuous paths, in and out of the plane of section, followed by the dendrites. The total dendritic lengths of 10 motoneurons (3 trapezius, 5 biventer cervicis-complexus, 2 splenius) ranged from 66,000 to 90,000 μm. A more detailed analysis of 8 motoneurons showed that the number of dendritic terminals per motoneuron was 85 to 124 and the average dendritic length from terminal to soma was 1,288 μm (range 1,149-1,582 μm, n = 792). These results confirm our previous measurements and show that the lengths of individual dendrites as well as the total dendritic length of neck muscle motoneurons are approximately 50% larger than large hindlimb muscle motoneurons. This increase in dendritic tree length was not matched by a corresponding increase in either average soma diameter (58.0 μm) or average primary dendritic diameter (5.6 μm). Indeed the soma diameter of neck and hindlimb muscle motoneurons were identical and the average diameter of primary dendrites of hindlimb muscle motoneurons was larger than that of neck muscle motoneurons. It was not surprising therefore to discover that the dendritic surface of neck muscle motoneurons was 420,000 μm^2 (n = 3), a value only 16% larger than similar measurements of large hindlimb motoneurons.

In summary, our results show that the motoneurons in the upper cervical spinal cord have unusually long dendrites but measurements of other parameters of dendritic tree size show comparable or smaller values than are reported for hindlimb motoneurons. (Supported by the Canadian MRC.)

284.4 PROJECTIONS OF LARGE AFFERENTS FROM LEG MUSCLES TO VARIOUS MOTONEURON POOLS IN MAN. C.C. Mao*, P. Ashby, D. McCrea*.

Our present understanding of the interpretation of poststimulus time histograms (PSTH) leads to the conclusion that one can infer the latency and shape of postsynaptic potentials from changes in the probability of firing of a neuron (Fetz and Gustafsson, Soc. Neurosci. Abs. 6:715, 1980). Using single motor unit recordings during small tonic voluntary muscle contractions one can build up a background of motoneuronal activity against which to test the effect of an electrical stimulus to a peripheral nerve upon the PSTH (eg. Stephens et al, Nature, 263:343, 1976 ; Ashby and Zilm, J. Neurol. Neurosurg. Psychiat. 41:684, 1977).

We have used this type of single motor unit analysis to examine the nature of the projections from the large diameter (low threshold) afferents in peripheral nerves of the lower limb to various species of human motoneurone. Based upon the assumption that the species of afferents excited by the lowest strength electrical stimuli are Ia muscle spindle afferents, we have examined the pattern of Ia excitation and inhibition in man in order to produce a picture of reflex pathways as has been done in the cat (Eccles et al., J. Physiol. 137:22, 1957; Eccles et al., J. Neurophysiol. 25:544, 1962) and the baboon (Lundberg, pers. comm.) as a preliminary step in our ongoing studies of human spasticity.

Increases in the probability of firing of homonymous motor units presumably caused by Ia EPSPs, were seen using electrical stimuli as low as .6 of the voltage required to elicit the smallest muscle contraction (M_T), but varied depending upon the nerve stimulated.

The overall patterns seem similar in the cat, baboon and man, however, in man the projections of medial gastrocnemius afferents to soleus motoneurons are weak. Low threshold afferents in the common peroneal nerve result in inhibition of medial gastrocnemius motoneurons, but facilitation of soleus motoneurons.

This research was supported by the Multiple Sclerosis Society of Canada and the Medical Research Council of Canada.

284.5 CHOLINERGIC AND NON-CHOLINERGIC PROPERTIES OF RENSHAW CELL FIELD POTENTIALS. J. Willetts* and W. G. VanMeter. Dept. Veterinary Physiology and Pharmacology, Veterinary Medicine Building, Iowa State University, Ames, IA 50011.

Effects of intravenous administration of selected drugs on Renshaw cell field potentials (RFP) evoked by antidromic stimulation (2Hz, 2x threshold) via the ventral root of lumbar segment 7 (L-7) of spinal cords of DIAL anesthetized cats were recorded by conventional methods from single barrel glass micropipettes (tip diameter 1.0-1.6μm, resistance 2.0-4.0Mhm) filled with 2.7M NaCl. Responses were observed during control stimulation (2Hz, 2x threshold) and in the presence of excess endogenous ACh modeled by repetitive stimulation at 20Hz for 10-60 seconds.

In control periods, RFP were inhibited after 30-60 seconds of 20Hz stimulation and recovered during the first 3 minutes after stimulation. In the presence of Eserine (50μg/kg, and 100μg/kg i.v.) RFP were inhibited in a dose dependent manner after 0-30 seconds of 20Hz stimulation. In addition, subsequent to 20Hz stimulation, RFP were reduced 10-12% in control animals and 17-20% in the presence of Eserine.

RFP were unaffected by Eserine (100μg/kg i.v.) during 2Hz stimulation, but the addition of atropine sulphate (0.5mg/kg i.v.) decreased the duration of RFP. The addition of mecamylamine (1.0 mg/kg i.v.) further reduced the duration of RFP. However, the initial two potentials of the RFP which occur in the first 5 msec. were still present 30 minutes after mecamylamine administration.

While amphetamine (0.5mg/kg i.v.) failed to enhance or antagonize the effects of a previously determined suboptimal dose of Eserine (20μg/kg i.v.), in doses of 0.5 and 1.0mg/kg i.v. it appeared to facilitate recovery of RFP after 20Hz stimulation.

The data indicate three components to the RFP; muscarinic, nicotinic and a third non-cholinergic one.

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284.6 ORGANIZATION OF PUDENDAL NERVE EFFERENTS AND PRIMARY AFFERENTS IN THE SPINAL CORD OF THE RHESUS MONKEY. J.R. Roppolo, I. Nadelhaft, W.C. deGroat. Dept. Pharmacol. and Neurosurgery, Univ. of Pittsburgh, and V.A. Medical Center, Pgh., PA 15261

The pudendal nerve is composed of afferent and efferent axons which innervate the muscles and skin of the perineum and pelvic floor. In the present experiments HRP tracing techniques were used to study the distribution of pudendal motor neurons and primary afferent neurons in the lumbosacral segments. Following application of HRP to the central cut end of the pudendal nerve of the Rhesus monkey, labelled motor neurons were located primarily in the S_1 and L_7 spinal segments. In transverse sections they appeared as an oval shaped group of cells (360 μ in diameter) at the base of the ipsilateral ventral horn, medial to the lateral motor nucleus. This group of cells termed Onuf's nucleus which contained an average of 418 medium size (52 X 31 μ) neurons, extended an average of 9.3 mm. rostrocaudally. In one of four animals the nucleus was split into two parts, a dorsal and ventral portion. In longitudinal section the nucleus appeared as a discontinuous column of cell clusters (260 X 200 μ) separated by areas (70 μ) which contain few or no labelled cells. These clusters were bridged by a prominent network of longitudinally running dendrites. Dendrites could also be seen in the transverse plane. One group of dendrites which extended dorsomedially ended in the dorsal commissure near the central canal, while a second group extended dorsolaterally to the intermediolateral gray, some following the border of the ventral horn.

An average of 8700 DRG cells were labelled in each monkey. Approximately 85% of these cells were located in a single DRG (S1 or S2) which was always located one spinal segment caudal to Onuf's nucleus. The density of afferent label in the spinal cord paralleled the numbers of labelled DRG cells in the corresponding segments. Afferent labelling in the dorsal columns and Lissauer's tract (LT) extended from S3 to at least L4. A prominent collateral fiber bundle passed medially over the apex of the dorsal horn (DH) to the dorsal commissure and across the midline for a short distance. Terminal fields related to this pathway could be seen in lamina I and II and bilaterally in the dorsal commissure. A much less prominent pathway passed along the lateral edge of the dorsal horn with a few collaterals passing medial in lamina VI to the dorsal commissure.

In summary, the pudendal nerve provides an extensive afferent input to the spinal cord, the major part of which is caudal to its efferent outflow. Afferent projections in the spinal cord extend into LT from which a major collateral pathway passes medially around the DH. This medial projection contrasts with the extensive lateral projection system characterizing visceral afferent pathways of the pelvic nerve.

284.7 AN ELECTRON MICROSCOPIC STUDY OF THE WHITE MATTER OF THE RAT SPINAL CORD. Kyungsoon Chung* and Richard E. Coggeshall, Marine Biomedical Institute and the Depts. of Anatomy and of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas.

The white matter of the spinal cord is thought to consist primarily of myelinated fibers. It is clear that there are an abundance of myelinated fibers in these pathways, but unmyelinated fibers have not been studied to any great extent. In the present study the lateral and ventral funiculi of the spinal white matter of the S_2 segment in the rat were examined in the electron microscope, which can resolve both myelinated and unmyelinated axons. Our preliminary data indicate that the unmyelinated axons outnumber the myelinated axons in the lateral funiculus and form a significant component of the ventral funiculus.

RAT 1

S_2 Segment

	LATERAL FUNICULUS	VENTRAL FUNICULUS
MYEL. FIBERS	50,057	20,492
UNMYEL. FIBERS	109,928	7,149

It is interesting that the myelinated fibers are distributed relatively uniformly throughout both funiculi, where as the unmyelinated fibers are most numerous in the dorsolateral fasiculus (where the unmyelinated/myelinated ratio is 10/1). Further counts and fiber diameter spectra will be presented at the meeting.

Supported by grants NS10161, NS17039 and NS11255 from the National Institute of Health.

284.8 PRIMARY AFFERENT INPUT TO THE DORSAL LATERAL FUNICULUS AND THE SACRAL PARASYMPATHETIC GREY OF THE CAT SPINAL CORD. J.C. Bresnahan, G.M. Mawe and M.S. Beattie. Dept. of Anat., Div. of Neurosurg. and Neurosci. Res. Lab., Ohio State Univ., Columbus, OH 43210.

Primary afferent input to the lumbosacral spinal cord of the cat has been examined by intraaxonal injury filling of dorsal roots, using techniques which allow for sequential light and electron microscopic analysis. Twenty hrs. following HRP application to cut dorsal rootlets, animals were perfused and the tissue processed using diaminobenzidine as the chromagen. Vibratome sections flat embedded between sheets of Aclar (Allied Chemical) allowed for light microscopic observation prior to sectioning for the electron microscope. This report will focus on the axonal trajectories of dorsal root axons entering the regions of the sacral parasympathetic nucleus (SPN) and into the dorsal lateral funiculus.

A fascicle of fibers descending along the lateral border of the dorsal horn distributes to the SPN. Swellings along these fibers appear to be in apposition to the somata of the SPN suggesting axo-somatic contacts. The main terminal field corresponds to the inner band region described by Nadelhaft et al. (1980) which is purported to contain interneurons and projection cells associated with sacral parasympathetic responses. Some of these fibers continue further ventrally to enter the lateral band region (Nadelhaft et al., 1980) containing the perikarya of the efferent neurons projecting to the bladder. In addition, fibers leave this lateral fascicle and distribute to the doral lateral funiculus, an area containing scattered neurons which is rich in enkephalin and substance P (Bresnahan et al., 1982; Glazer and Basbaum, 1981; and others). These fibers to the dorsal lateral funiculus are not seen in any appreciable quantity at lumbar levels in either cat or rat, both of which have cell bodies in this region of the white matter. This projection to the dorsal lateral funiculus in the sacral cord has been noted after application of HRP to the pelvic nerve in cat (Morgan et al., 1981) suggesting that some of these fibers are visceral afferents. Electron microscopic studies are currently underway to evaluate the ultrastructural characteristics of these projections. (Supported by NS-14457 and NS-10165.)

284.9 INTRACELLULAR STAINING OF γ-AMINOBUTYRIC ACID-RESPONSIVE CELLS IN RAT SPINAL DORSAL HORN IN VITRO. V. Nedeljkov* and M. Randić. Dept. of Vet. Physiol. Pharmacol., Iowa State University, Ames, IA 50011.

Immunocytochemical studies have demonstrated presence of glutamic acid decarboxylase (GAD)-positive terminals on rat dorsal horn neurons. There is also some evidence for γ-aminobutyric acid (GABA)-mediated postsynaptic inhibition in the adult spinal cord. Since the knowledge about identity of dorsal horn neurons-containing GABA receptors is as yet not available, we used the method of intracellular staining with horseradish peroxidase (HRP) to examine the morphology and localization of the GABA-responsive cells.

Rats 9 to 12 days old were used. After lumbosacral laminectomy 300μm thick horizontal dorsal horn slice was maintained in an oxygenated Ringer solution according to the recently described technique (Murase et al., Brain Res., 234:170-176, 1982). HRP was injected intracellularly into the dorsal horn neurons by depolarizing 200 msec pulses of 5-15 nA, applied at 1/sec for 2-4 minutes through the micropipettes filled with 4% HRP in 0.2M KCl, buffered at pH 7.6. Microelectrodes with tip diameters of < 0.5μm, having DC resistances of 60-150 MΩ were used. Following incubation of 1-2 hrs, and overnight fixation, the slices were processed with diaminobenzidine. Labeled cells were photographed or traced with a camera lucida.

The pharmacological and morphological analysis is based on data obtained from 15 well-stained dorsal horn neurons. In the presence of tetrodotoxin (10^{-6}M), bath-application of GABA (10^{-3}M) produced a reversible hyperpolarization, which was associated with a fall in neuronal input resistance. However, larger doses of GABA (5×10^{-3}-10^{-2}M) frequently evoked a biphasic response whereby the initial hyperpolarization was followed by a depolarization. Both responses were associated with a fall in neuronal input resistance. The GABA-responsive neurons were localized at all levels of the spinal dorsal horn. They vary considerably in size, shape and dendritic arborization. The cell bodies range from fusiform to polygonal with soma diameters from 7 x 12μm to 15 x 35μm. Their dendritic processes could be followed for up to 750μm in the medio-lateral direction and for 450μm along the rostro-caudal axis. HRP staining reveals frequent dendritic swellings and does show in some cells presence of dendritic spines.

Our results demonstrate a remarkable morphological heterogeneity of the GABA-responsive immature rat dorsal horn neurons. It appears also that the rat spinal cord slice preparation may serve as a good model for combined pharmacological and morphological studies of spinal neurons.

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284.10 A NEW TECHNIQUE FOR INTRAOPERATIVE SPINAL CORD MONITORING. J.T. Molt, H. Kushner,* and R. Blumenstein.* Dept. of Physiology and Dept. Anatomy. Hahnemann Medical College, Philadelphia, PA 19102

The most widely employed method of intraoperative spinal cord monitoring is the sensory evoked potential (SEP) which results from activity that is conveyed primarily in the dorsal column pathways. A major problem with this technique is its inability to monitor descending long tract conduction. To assess the integrity of descending pathways, we are suggesting the use of the spontaneous spinal electrogram (SEG) as an intraoperative monitor. The SEG consists of large (150 μV), slow (30 msec at the base), random, negative voltage fluctuations. The occurrence of the slow waves increases in the spinal cord caudal to a surgical transection indicating an inhibitory effect of descending input. The specific aim of this study was to show that the SEG could be used as a measure of disruption of intact spinal cord pathways during surgery. It was hypothesized that surgical interruption of descending pathways would cause a quantifiable change in the SEG caudal to the site of interruption when compared to the SEG recorded from a rostral site. To test this, the SEG was recorded from two sites on the exposed dura of the cord overlying L2 and L4 in anesthetized cats. The following surgical manipulations were performed on different groups of animals at L3: (1) midline myelotomy (2) midline myelotomy with removal of grey matter (3) midline myelotomy with removal of grey matter and a narrow circumferential band of white matter (4) midline myelotomy with removal of grey matter and a wider band of circumferential white matter. The SEG was subjected to on-line spectral analysis. Following a two week period the animals were assessed using a neurological exam, anesthetized and perfused. The surgically traumatized section of cord was removed for histological assessment. The experiment yielded the following numerical data: clinical score, change in slow wave occurrence as a percentage, shift in spectral frequency and amplitude as percentages, and cross sectional area of injury as a percentage. The data were subjected to multiple regression analysis. The results can be summarized as follows: midline myelotomy or midline myelotomy plus removal of grey matter did not significantly affect the SEG or the cat's behavioral performance. As more white matter was surgically removed a significant correlation appeared between the amount removed and changes in the SEG both in terms of spectral changes and changes in frequency of occurrence of the slow waves. These changes also showed a significant correlation with the behavioral score of the animal. This indicates that the SEG may be a quantifiable determinant of descending pathways that can be used as an intraoperative monitor of cord function. Supported by Biomed. Res. Support Grant 5-S07-RR05413.

284.11 FINE STRUCTURAL CHANGES IN L-7 (VII) IN DFP TREATED CATS. K. C. Sikora-VanMeter, W. G. VanMeter, J. Willetts* and P. A. Grieve*. Dept. of Vet. Physiology and Pharmacology, Vet. Medicine Bldg., Iowa State Univ., Ames, IA 50011.

The fine structural changes in spinal segment Lumbar 7, Lamina VII (L-7(VII)) have been studied in cats after single and chronic (14 days) low dose (0.1-0.75 Mg/Kg S.C.) exposure to diisopropylfluorophosphate (DFP).

Motoneurons of the chronically treated animals showed a greater number of lysosomes, neurofilaments and vesicle-like structures. An increased amount of glycogen granules within the motoneurons as well as coated vesicles within axon terminals was observed in both acute and chronically treated animals. Both changes were more pronounced after chronic DFP exposure. Also, morphological evidence of axon and terminal degeneration was only seen in chronically treated animals. Degeneration within axons and presynaptic terminals is revealed by the presence of membrane bound structures, glycogen granules, increased amounts of smooth axonal endoplasmic reticulum and an accumulation of microtubules. In addition, a decrease in synaptic vesicles was occasionally observed in the terminals.

In conclusion, a single low dose (0.1 Mg/Kg or 0.7 Mg/Kg S.C.) failed to give morphological evidence of increased toxicity, while chronic administration to cumulative amounts less than LD50 (1.4 Mg/Kg S.C.) or greater than LD50 (10.5 Mg/Kg S.C.) did.

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284.12 REGIONAL BLOOD FLOW RATES DURING FOCAL SPINAL CORD ISCHEMIA. J. A. Zivin. Univ. of Mass. Med. School, Worcester, MA 01605.

The pathophysiology of central nervous system infarction can be studied by the use of a rabbit spinal cord ischemia model. For such studies, it is necessary to know the blood flow rates at many points along the cord, and it is particularly important to use a regional blood flow measurement method that is accurate at very low flow rates. Therefore, a diffusible tracer technique was chosen.

For 20 min, unanesthetized rabbits had their aortas occluded just below the renal arteries by a snare ligature. Over 1 min. ^{14}C-iodoantypyrine was injected intravenously and sequential arterial samples were collected. The blood flow to the spinal cord was then abruptly terminated by severing the aorta at a high abdominal level with a previously implanted ligature. The radioactive tracer concentrations in blood and serial sections of spinal cord were measured, and the blood flow rates were calculated.

It is possible to clearly identify the location of the ischemic tissue which extends from the upper lumbar segments to the caudal end of the spinal cord. The flow rates rostral to the ischemic area averaged 20.3 ± 1.9 ml/100g/min (mean ± s.e, n = 6) which is normal for rabbits. The transition zone between the normally perfused and ischemic regions averages 2.2 cm in length. The flow in the most ischemic region is 0.39 ± 0.13 ml/100g/min (n = 3) which is 1.9% of normal. There was no difference in flow rates between gray and white matter in the ischemic zone.

Thus, use of these methods allows detailed analysis of spinal cord blood flow during ischemia. It was demonstrated that some flow persists even in the most ischemic spinal cord regions 20 minutes after the onset of occlusion of the aorta. Finally, the severe gray matter necrosis with preservation of white matter which ultimately develops as a consequence of this type of ischemia is caused by differential succeptibility to ischemia of gray versus white matter rather than differences in blood supply.

(Supported by PHS grants NS 00456 and NS 15827)

284.13 ULTRASTRUCTURAL ALTERATIONS IN FELINE SPINAL CORD PRODUCED BY FERROUS CHLORIDE. E.D.Means,D.K.Anderson & R.Fitzgerald*. Spinal Cord Injury Lab.,VAMC & Univ. of Cinti.,Col. of Med., OH. 45220.

Experimental spinal cord injury (SCI) is characterized by hemorrhagic necrosis of gray & white matter. It has been suggested that iron & other components of blood are catalysts of free radical induced lipid peroxidation. Moreover, iron has been implicated as a catalyst of free radical induced injury in other neurological diseases. To study the ultrastructural effects of free radical induced injury on the SC, 5µL of 100mM $FeCl_2$ was injected into the feline SC gray matter at the L-4 segment using a 29 gauge needle. At ½hr (N=2), 1hr (N=2), 4hr (N=2), 8hr (N=2), & 24hr (N=4) after injection, the animals were sacrificed using a formaldehyde-gluteraldehyde sequence. Two animals were sacrificed at 24hrs following the intraspinal injection of saline (5µL). The SC was removed & 1-2mm transverse sections were postfixed in osmium tetroxide, stained with uranyl acetate & lead citrate & embedded in spurr. Whole transverse 1µ sections were used for orientation prior to thin sectioning.

At ½hr, $FeCl_2$ injected tissue showed: 1) electron dense deposits on the luminal surface of blood vessels, on platelets & platelet-fibrin thrombi, 2) electron dense axoplasm containing "empty" mitochondria while other mitochondria showed electron dense particles within cristae, 3) neuronal cytoplasm containing numerous degenerating mitochondria & irregularly dispersed RER within a granular-amorphous matrix, 4) random clumps of degenerating myelin. One hour material displayed similar changes except for the cytoplasm of neuronal somata & dendrites which showed marked increase in electron density; few elements save for empty mitochondria were identifiable. Occasional terminal boutons were also electron dense. Similar alterations appeared in the SC of 2hr animals. In addition, particulate, electron dense deposits appeared in the basal lamina of blood vessels. Neutrophils were present in the neuropil of SC gray matter in 8 & 24hr animals. At the same time periods, numerous platelet-fibrin thrombi were observed at times extruding through the vessel wall. Also noted were electron dense deposits on myelin & neuronal membranes & prominent vesicular degeneration of myelin. The SC of $FeCl_2$ injected animals showed only minor changes which were dissimilar to the alterations seen in the $FeCl_2$ animals.

These ultrastructural data show that $FeCl_2$ induced lipid peroxidation produced the following prominent changes in feline SC: 1) thrombosis of vessels, 2) inflammation, 3) dense degeneration of neuronal cytoplasm with early mitochondrial change, 4) vesicular degeneration of myelin. This information is consonant with the biochemical data from our laboratory.

284.14 ACTIVITY OF FELINE SPINAL CORD ATPASE'S AND SUCCINATE DEHYDROGENASE AFTER IN VIVO INJECTION OF $FeCl_2$. D.K.Anderson, E.D. Means, K.R. Wagner, E.S. Green* and T.R. Waters*. Spinal Cord Injury Lab. VAMC and Univ. of Cinti. Col. of Med., Cinti., OH 45220

Evidence is accumulating indicating the involvement of free radical induced lipid peroxidation of neuronal membranes in the autodestruction of spinal cord (SC) tissue subsequent to trauma. The presence of focal hemorrhage in SC tissue following injury may produce an iron overload. Since iron is known to catalyze free radical formation, we decided to determine the effect of $FeCl_2$ on neuronal plasma and mitochondrial membranes utilizing enzymatic markers. Cats were anesthetized with pentobarbital, intubated and placed in a spinal stereotoxic frame. The L4 SC segment was exposed, the dura incised, and a 29 gauge needle stereotoxically placed in each anterior horn. Five µl of 100mM $FeCl_2$ was injected into each anterior horn. Controls were injected with similar volumes of 0.9% NaCl. At either 2, 8, or 24 hrs postinjection, the L4 segment was frozen in situ and removed. In a cold room, gray and white matter was separated and the gray core was homogenized and analyzed for Na^+-K^+- and Mg^{++}-ATPase activity. Succinate dehydrogenase (SDH) activity was measured at 24 hrs postinjection. Na^+-K^+-ATPase activity in $FeCl_2$ injected SC's was 34%, 60%, and 38% and Mg^{++}-ATPase activity was 82%, 39%, and 42% of NaCl injected controls at 2,8 and 24 hrs postinjection, respectively. At 24 hrs, SDH activity was 56% of control. The partial loss of enzymatic activity is consistent with the ultrastructural damage to neuronal plasma membranes, mitochondria, and other subcellular organelles demonstrated in our laboratory following injection of $FeCl_2$. These data indicate that $FeCl_2$ has a detrimental effect on the activity of cellular and mitochondrial membrane bound enzymes that may be the result of an iron catalyzed free radical induced destructive peroxidation of membrane lipids. The importance of iron as a pro-oxidant in traumatized spinal cord tissue remains to be determined.

284.15 SUPERSENSITIVITY TO NOREPINEPHRINE AND SEROTONIN IN THE INTERMEDIOLATERAL CELL COLUMN FOLLOWING CHRONIC SPINAL TRANSECTION. Leslie P. Felpel and Ronald D. Huffman, Dept. Pharmacology, U. Texas Health Science Center, San Antonio, TX 78284

Autonomic hyperreflexia, characterized by abnormally high blood pressure fluctuations, headache, bradycardia, and sweating, occurs in approximately 85% of quadraplegic patients. Stimuli producing autonomic hyperreflexia most commonly arise from distention of the hollow viscera, muscle spasm, or other visceral stimuli. Though the cause of autonomic hyperreflexia is unknown, there are data which suggest that neuronal hypersensitivity could be a contributing factor. The aim of the present study was to test whether autonomic neurons in the intermediolateral (IML) cell column, below the level of transection, develop supersensitivity to putative neurotransmitters following denervation (spinal transection). Adult female cats anesthetized with pentobarbital (35 mg/kg), were either spinal transected or sham operated at the third thoracic (T3) spinal level. Two to three weeks following the operation, the animals were anesthetized with alpha chloralose (40 mg/kg) and urethane (800 mg/kg) and all surgical procedures performed. Single IML units were recorded at the T5 or T6 level with either single or 7 barrel micropipettes and were identified as either antidromic or orthodromic, depending upon their response to splanchnic nerve stimulation.

Forty units were recorded in acute or sham operated control animals (n=10), twelve of which were pharmacologically investigated. In chronic spinal-transected animals (n=8), twenty-six units were recorded, fourteen of which were pharmacologically investigated. Two to three weeks following spinal transection, the inhibitory response of spontaneous or d,l-homocysteic acid-driven IML units to 1-15 nanoamperes (nA) of both norepinephrine and serotonin was significantly ($p<0.05$) increased over that of controls (35.8% vs 12.9% respectively for norepinephrine; 25.6% vs 0.0% respectively for serotonin). When higher levels of ejection current were used (20-160 nA), no significant difference was observed in the two groups of animals. The inhibitory effect of GABA (1-160 nA) on IML units did not differ in control vs. spinal transected animals. The mean spontaneous activity of IML neurons recorded in control (3.1 Hz, range 0-20) and spinal transected (3.5 Hz, range 0-15) animals did not differ.

These results suggest that following chronic spinal transection, IML neurons become hypersensitive to at least two putative neurotransmitters, norepinephrine and serotonin, both of which are known to be present within the IML cell column. (Supported by the Biomedical Research Grant Program, Division of Research Resources, NIH.)

284.16 ULTRASTRUCTURAL FEATURES OF MOTOR NEURONS AND MICROVASCULATURE IN REXED AREA IX OF THE RAT SPINAL CORD AFTER CONTUSION AND DMSO TREATMENT. P. K. Hill, J. C. de la Torre, D. L. Bullock*, S. M. Thompson* and M. L. Beckett*. Eastern Va. Med. Sch., Norfolk, VA 23501 and Northwestern Univ., Chicago, IL 60611.

The project was conducted to elucidate the ultrastructural features of the adult rat Rexed area IX motor neurons and microvasculature following acute injury and dimethyl sulfoxide (DMSO) treatment. Three groups of animals were included in the study: Group I received L-1 laminectomy, no contusion and four days of saline-filled mini-pump implantation. Group II received L-1 laminectomy, contusion of L1-2 with a force of 0.18 Newtons and four days of saline-filled Alza mini-pump implantation. Group III received L-1 laminectomy, contusion of L1-2 with a force of 0.18 Newtons and four days of DMSO-filled mini-pump implantation. Group II and III animals also received an intraperitoneal injection of saline and 1.5 gm/kgm body weight of DMSO (50%), respectively, three times daily for four days in order to maintain systemic levels of the drugs. Qualitative ultrastructural examination of the right and left Rexed IX areas was performed at the lesion site (L1-2) as well as 1 centimeter proximal (T11-13) and distal (L3-5).

Motor neurons from all three levels of Group III showed greater intracellular and extracellular preservation than identical areas of Group II. The area distal to the lesion site in Group III demonstrated the greatest degree of preservation of any level in either Group II or III. The microvasculature of Rexed area IX at all three levels in Group III showed fewer pathological changes than comparable areas of Group II.

If the microvasculature of Rexed area IX can be preserved by DMSO during the early phases of spinal cord injury and allowed to maintain a homeostatic supply of oxygen and nutrients to the damaged tissue, the possibility of sparing traumatized motor neurons and surrounding terminals from irreversible degenerative changes is significantly increased.

Sponsored by Eastern Paralyzed Veterans Association

284.17 OXIDATIVE METABOLISM AND ULTRASTRUCTURAL CHANGES FOLLOWING IMPACT CORD INJURY - COMPARISON WITH STRETCH INJURY, Shokei Yamada, M.D., Robert L. Schultz, Ph.D., Lloyd Dayes, M.D. & David Knierim, M.D., Loma Linda University, School of Medicine, Section of Neurosurgery, Loma Linda, California, 92350

Impact spinal cord injuries are known to result in progressive necrosis in the central cord. This progression has been attributed to low oxygen tension, diminished spinal cord blood flow, or increased catacholamine in the cord. However, early architectural changes have not been thoroughly described, particularly with regard to the ultrastructural studies.

The authors produced impact injury to the lumbosacral cord of cats using the method described by Freeman & Wright. Spinal cords were perfused immediately after the impact injury for ulstrastructural studies. The forces of impact consisted of 20, 50, 100, 200, 300, and 400 gm-cm.

In the 100 gm-cm impact cord, a significant number of membrane breaks were noted in the neuronal and glial cells. In 200 or 300 gm-cm impact cords, neuronal and glial damages were noted, associated with extracellular edema. Swollen degenerated mitochondria were frequently found. In 400 gm-cm impact cords, many neurons displayed dark discoloration (indicating dying neurons), many myelin figures were present, and intracellular as well as extracellular edema was noted in many areas.

Reviewing our reflection-spectrophotometric studies on traumatized cords with 20 gm-cm impact and 3 gm traction (stretch injury), cytochrome a,a_3 was found to be mildly reduced. However, cytochromes were highly reduced in 5 gm traction and 100 gm-cm impact cords.

In conclusion, mild impact or traction cord injury which is not associated with ultrastructural change has already shown mild impairment of oxidative metabolism. The impact or traction injury which is associated with multiple membrane breaks of neuronal and glial cells results in severe impairment of oxidative metabolism, although these changes could be reversible. The impact injury which is associated with destructive neuronal changes (with some neurons dying) may progress to more extensive necrosis.

284.18 CERVICAL CORD FIBER DEGENERATION FOLLOWING EXPERIMENTAL CONCUSSION IN THE RAT. Mary D. Guthrie and L. Claire Parsons. Department of Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, Texas, 78284, and Department of Physiology, University of Virginia Medical School, Charlottesville, Virginia, 22901.

Twenty-eight adult hooded rats were used to study the degenerating fibers in the cervical spinal cord following experimental cerebral concussion (ECC). In each animal the calvarium was reinforced with a plastic disc to prevent skull fracture. A blow of twenty-six to forty PSI was delivered by a fixed spring coil concussion gun to twenty-four of the animals; four animals received sham blows and were used for controls. Ten of the concussed rats were administered a second blow seven days after the first blow; and five of that group received a third blow one week after the second blow. Half of the animals were killed three days post ECC; the other half were killed seven days ECC. Sections 25 μm in thickness were made of the cervical cord. Every twenty-fourth section was stained with Fink-Heimer modification of the Nauta-Gygax silver staining method; and for each of these a contiguous section was stained with the Nissl method. Terminal degeneration was found in the posterior horn of 17% of the animals, while twice as many animals showed terminal degeneration in the anterior horn. Our previous study of the brain following ECC showed such extensive areas of degeneration, it is not surprising that forty-seven percent of these animals demonstrated degenerating fibers in the anterior and lateral columns of the cord; i.e., antegrade degeneration. It is of interest that sixty-five percent of the animals had extensive degeneration in the posterior column. Since the majority of posterior column fibers are sensory and from dorsal root ganglion cells which are not injured by the ECC, this degeneration is retrograde. This is of clinical significance in explaining marked sensory functional deficit following brain concussion. (Supported by NIH Biomedical Research Support Grant RR05654 and Grant-in-Aid from University of Virginia School of Medicine.)

284.19 Suprasegmentally Induced Motor Unit Activity in Paralyzed Muscles - Effects of Reinforcement Maneuvers. M.R. Dimitrijevic, M.M. Dimitrijevic*, W.B. McKay*, and A. M. Sherwood.

In spinal cord injury patients with established spastic paraplegia, reinforcement maneuvers (RM) elicit two types of activity in paralyzed legs. To characterize RM-elicited muscle activation, we examined 58 patients with stabilized spinal cord lesions. They had no volitional activation of leg muscles, but had preserved segmental reflexes.

Using multichannel surface EMG recordings, a series of maneuvers were undertaken to assess the patient's reflex and involuntary functioning. The RM's consisted of: breath holding, (or Valsalva maneuver), isometric neck flexion with resistance applied manually to the forehead, the Jendrassik maneuver, right hand grip and left hand grip.

Two distinctly different types of responses could be seen in EMG recordings from the legs. The first type, termed R1, was of low amplitude (30-50 microvolts), and relatively short response time (0.8-1.2 S), and appeared in 1-3 muscles, whereas the second type, termed R2, appeared in 8-10 (i.e., all measured) muscles, had a response time closer to 2 to 3 S, and an amplitude in the 150-200 microvolt range. On many occasions, the low amplitude, R1 response was followed by the second type of response, termed the R2 response, which is much like a spasm. The R2 response EMG was of relatively large amplitude and was present in several muscle groups, usually including most of the muscles of the leg, and quite frequently spreading bilaterally. The R2 response could occur following an R1 response, or could occur independently. The R2 response time was much longer, and somewhat more variable than was the R1 response.

RM's elicited EMG activity in paralyzed muscles in 39 of 58 patients. In addition, vibration of the quadriceps femoris muscle revealed one additional patient who could elicit some EMG activity through co-activation of RM and vibration.

We have found that SCI patients with preserved segmental reflexes may or may not show evidence of descending facilitatory pathways. In those which do show such evidence, this is a clear indication of surviving or regenerated fibers passing through the lesion. In such cases with minimal surviving fibers, it may be possible for patients to initiate activity, but not to control the amplitude or duration of the response. Activation may be of isolated, local motor control centers in the spinal cord, or may result in initiation of activity in the entire segmental interneurone system, with consequent suprasegmentally induced flexor reflex activity of paralyzed legs.

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285.1 MONITORING ENERGY METABOLISM IN THE WAKING BRAIN BY SURFACE COIL - NUCLEAR MAGNETIC RESONANCE (SC-NMR). Ruthmary K. Deuel, David M. Schickner, Genevive M. Yue, and Joseph J. H. Ackerman. Dept. of Ped., Chem., and Med., & the McDonnell Center for Studies of Higher Brain Function, Washington University, St. Louis, MO.

Recently, P-31 SC-NMR has permitted localized observation of intracellular sugar phosphate (SP), inorganic phosphorus (Pi), phosphocreatine (PCr), ATP, and pH through the intact integument. As central neural metabolic events vary widely during normal waking behavior, SC-NMR is ideal to study them. Relative immobility of subjects, however, is required.

250 gm Sprague-Dawley rats were adapted with initial handling, then daily practice sessions in the vertical position required by the NMR spectrometer. At the start of each session, rats were anesthetized with halothane, secured with a tooth bar in a magnet-compatible plastic restraint, and a surface coil (SC) fitted against the head. When the rat responded to pinprick, the restraint was placed upright. Animals that adapted readily to long periods of relative immobility in the upright position formed the study group. Prior to actual spectroscopy, rats were fasted for 16 hours and tail vein catheters placed. Patency was verified (and final restraint placement facilitated) by response to a bolus of 20 mg/kg of pentobarbital delivered through the catheter. Consciousness and position vis-a-vis the SC were checked in the vertical position prior to insertion into a Brucker WH-360 NMR spectrometer. Spectra displaying baseline cerebral phosphate metabolites were collected for 20 min before an I.V. bolus of 2-deoxyglucose (2-DG, 500 mg/kg) was injected. Baseline pH was determined from the chemical shift of Pi.

After injection of 2-DG (that can only be detected in its phosphorylated, intracellular form by P-31 MNR), the SP rose to maximum, more than double the control, at a mean of 55 min, followed by a rapid decline of SP over the next 30 min. Mean SP returned to baseline by 240 min. Controls (given 500 mg/kg of dextrose, or uninjected), showed inconsistent minor fluctuations in SP. Intracellular pH was stable throughout in both DG and control animals. At 240 min after injection, before animals were returned to their cages, blood glucose was measured. A mean rise of 200% was seen in DG animals, vs 15% in controls.

Cerebral intracellular metabolic events were monitored during the waking state in adapted rats by P-31 SC-NMR spectroscopy. Intracellular phosphorylation, followed by elution within four hours of injection of 2-DG,was observed.

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285.2 EFFECTS OF SLICE THICKNESS AND METHOD OF PREPARATION ON ENERGY METABOLISM IN THE IN VITRO HIPPOCAMPUS. W. D. Lust, T. S. Whittingham and J. V. Passonneau. Laboratory of Neurochemistry, NINCDS, NIH, Bethesda, MD. 20205

We previously reported some of the metabolic changes occurring in hippocampal slices during the initial 30 min of incubation following decapitation (Whittingham et al., Soc. Neurosci. Abs.:7, 1981). We have now expanded our observations to include the effects of 3 different conditions for slice preparation and of slice thickness on the metabolic profiles during an 8 hours of in vitro superfusion. Adult guinea pigs were decapitated and the hippocampi rapidly removed (1.5 min). In one group, the hippocampi and subsequent slices (0.5 mm) were then maintained in one of the following media prior to transfer into the incubation chamber (7 min): 1) bicarbonate buffer (ACSF) devoid of glucose and oxygen, 36°C; 2) oxygenated ACSF containing 4 mM glucose, 36°C; or 3) chilled oxygenated ACSF containing 4 mM glucose. Individual slices were removed periodically during the ensuing 8 hour incubation and were frozen rapidly in liquid nitrogen. Samples were assayed for adenylates (ATP, ADP and AMP), phosphocreatine (PCr), creatine (Cr), lactate and the cyclic nucleotides (cAMP and cGMP). For all three methods of preparation, the metabolites recovered to near steady-state levels within one hour of incubation. Steady-state levels of the adenylates, PCr, Cr and the cyclic nucleotides were approximately 50% of the in vivo concentrations, while those for lactate were elevated two-fold compared to the in situ guinea pig hippocampus. Despite the metabolic similarity among the three groups, the recovery of the evoked orthodromic responses in the dentate gyrus was compromised in slices prepared in medium #1. The peak spike magnitude of this group was 0.8 mV compared to 4.2 mV for slices prepared in media #2 and #3. There is no indication of metabolic failure during the entire course of an 8 hour experiment; the 8 hour metabolite values being the same or improved from those present at 4 hours. In a second group of experiments, the tissue was placed in medium #3 above and cut to 0.25, 0.50, 0.75, 1.0 and 2.0 mm thickness using a Sorval tissue chopper and incubated as described. Slice thicknesses from 0.5 to 1 mm exhibited similar metabolite profiles, while 2 mm thick slices were energetically depressed (energy charge of 0.76 compared to 0.89 for 0.75 mm slices), suggesting the presence of a significant anoxic core. The addition of 0.003% hydrogen peroxide, previously shown to improve in vitro viability (Llinas et al., Fed. Proc. 40: 2240,, 1981), did not improve the metabolite profile in the 2 mm slices.

285.3 THE DOSE DEPENDENT EFFECTS OF CREATINE AND CYCLOCREATINE ON SYNAPTIC TRANSMISSION IN THE DENTATE GYRUS DURING ANOXIA. T.S. Whittingham, W. D. Lust and J.V. Passonneau, Laboratory of Neurochemistry, NINCDS, NIH, Bethesda, MD. 20204

Previous work has shown that the addition of 25 mM creatine (Cr) to artificial cerebrospinal fluid (ACSF) produces a time-dependent rise in hippocampal slice phosphocreatine (PCr) content, and also greatly prolongs synaptic transmission in the dentate gyrus during anoxia (Whittingham and Lipton, Soc. Neurosci. Abs.: 6, 1980). We are currently investigating the effects of varying concentrations of creatine and of the cyclic analogue, cyclocreatine (CC), on metabolite concentrations and synaptic transmission. The effects of guanidoacetic acid (GA), a creatine precursor, were also investigated. Hippocampal slices were prepared in chilled oxygenated bicarbonate medium (ACSF), containing 4 mM glucose and incubated for one hour at 36.5°C prior to any manipulations. One set of slices were made anoxic at hourly intervals by superfusion with ACSF plus glucose and equilibrated with 95% nitrogen:5% carbon dioxide. Field potentials were monitored in the dentate gyrus following stimulation of the perforant path axons. After the second anoxic period, the experimental agents were added individually to the superfusion medium. For the measurement of the metabolites, the hippocampal slices were either 1) incubated under normoxic conditions for the entire 8 hr or 2) periodically exposed to brief periods of anoxia. Slices from both groups were removed at hourly intervals and were frozen in liquid nitrogen. There was little or no difference in metabolite levels between the two groups. After a 5 hr exposure to 5 and 25 mM Cr, synaptic transmission was 47 and 94% longer, respectively, than the values for the pretreated slices. The corresponding PCr levels were increase by 250 and 350%, while ATP concentrations were unaffected. The effects of 5 and 25 mM CC were biphasic, prolonging transmission by 93 and 100% at 1 hr, but by only 43 and 25% at 5 hr. There was a decrease in ATP content during the 5 hr of exposure to 25 mM CC, from 10 to 2 nmole/mg protein. CC also appeared to act as a convulsant; the seizure discharges were prolonged by 1) anoxia and 2) longer exposure to CC. GA (5mM) eliminated the population spike in normoxic conditions and halved the time to abolish transmission during anoxia. The results show that there is a more rapid and greater accumulation of PCr with increasing concentrations of extracellular Cr. Both Cr and CC prolong electrical responsiveness during anoxia, while GA appears to be detrimental. The protective effect of CC appears to be transient which may reflect a dual role; the apparent sparing of PCr stores on one hand and and its convulsant activity on the other.

285.4 pH transients in the brain cell microenvironment. R.P. Kraig, C. Nicholson, & C.R. Ferreira-Filho*. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016

pH transients in the extracellular brain microenvironment (pH_o) are important indicators of functionally significant changes in other extracellular variables. pH can be defined by the concentration of the strong ion difference, total weak acid concentration, and PCO_2. Hence, changes in pH_o solely reflect changes in these three independent variables, which relate directly to brain cell microphysiology. pH_o fluctuations themselves, however, are unlikely to directly influence brain function because of the paucity of enzymes present extracellularly and the relative pH_o insensitivity of ion channels and receptors.

pH_o and extracellular potassium concentration ($[K^+]_o$) were simultaneously measured in the cerebellar cortex of the anesthetized and spontaneously breathing rat using liquid membrane ion selective microelectrodes during stimulus evoked neuronal activity, elevated $[K^+]_o$, spreading depression (SD), and complete ischemia.

A train of local surface stimuli produced an alkaline shift in pH_o from a baseline of 7.30 to 7.35 followed by a long lasting acid phase that reached a plateau of 7.05-7.15 after 64s. Acidification was related to stimulus frequency, intensity, and duration. Superfusion with Mn^{2+} Ringer abolished the alkaline shifts while enhancing acid shifts. Superfusion of the cerebellar cortex with Ringer containing 30mM of K^+ progressively acidified pH_o to a plateau of 6.95-7.05. The acidification occurred in the presence of ouabainbut was reversed upon return to normal $[K^+]_o$ or with the addition of the glycolytic blocker, fluoride. Stimulus evoked alkaline shifts were enhanced by K^+ Ringer superfusion. Elevation of $[K^+]_o$ above 8-12mM often produced a spontaneous SD. Under these conditions the SD consisted of a pronounced alkaline transient followed by a small, long lasting acid shift. When SD was induced while baseline $[K^+]_o$ was normal, the alkaline shift was reduced and the acid shift enhanced. Complete ischemia began with a progressive acidification of pH_o and rise in $[K^+]_o$. When the latter reached 12mM, a second more rapid rise in $[K^+]_o$ occurred which was correlated with an alkaline shift similar to that seen during SD. pH_o eventually reached a plateau of 6.60-6.80. Superfusion with Ringer containing acetazolamide enhanced the amplitude but not the time course of all induced pH_o changes.

These results will be discussed in terms of the extracellular strong ion difference, total weak acid concentration, and PCO_2. We conclude that increased neuronal activity produces acid shifts as a result of the metabolic production of some anion, perhaps lactate. Alkaline shifts result from a shrinkage of extracellular space or release of an alkalinizing substance from cells. Finally carbonic anhydrase inhibition has an immediate and pronounced influence on pH_o homeostasis. (Supported by NS-03346 & NS-13742).

285.5 THE USE OF NEUTRAL RED AS AN INDICATOR OF INTRACELLULAR pH IN RAT BRAIN CORTEX IN VIVO. J. C. LaManna and K. McCracken*. Dept. Neurology, Sch. Med. Case Western Reserve Univ., Cleveland, OH 44106.

Control of intracellular pH is of great importance especially under conditions of metabolic stress. However, brain cortical pH changes are difficult to determine in the intact animal. We have evaluated the pH indicator neutral red (NR) for possible use as an in vivo pHi indicator. NR was administered i.p. to a series of rats. These rats were killed at 30, 60, 90, 180, and 360 minutes after dye administration. Cortical samples weighing about 300 mg were homogenized in .1 N NaOH and then mixed with 3 cc of octanol. After centrifugation, the octanol phase was removed and the NR concentration was determined spectrophotometrically against a tissue blank. Known amount of NR were added to undyed cortical samples at the time of homogenization to determine the recovery of the extraction procedure (about 50%). The mean content of dye found was 48, 20, 23, 11, and 10 ug/g (10 ug/g=35 uM) at the above time periods respectively. In a second series of experiments, the pK for absorption at the base peak and acid peak wavelengths were determined by reflection spectrophotometry. At concentrations of 10-100 uM, the pK was found to be 6.55 ± .03 in saline. In whole rat brain homogenates, concentrations of 10-77 uM, the pK was 7.06 ± .10. Thus, there was a significant ($p < 0.001$) of the pK to more basic pH by .51 units, which was not dependent on NR concentration over the range used.

In one rat, the reflected absorption spectrum was monitored directly from the exposed dural surface while NR was administered (i.p.). The change at the acid wavelength, when the pre-dye spectrum was compared to a spectrum recorded 2 hours post-dye, was about .09 OD units. From the homogenate standard curve data this corresponds to a concentration of 21 uM. The subsequent octanol-extracted concentration was determined to be 53 uM. This difference can be attributed to difference in penetration depth of the light in intact brain compared to homogenates.

Thus, it was possible to obtain concentrations of NR in rat cortex after single i.p. administration which were measurable by reflectance spectrophotometry. NR concentrations were constant 2 hours post-injection and persisted for at least 6 hours. From titration curves in brain homogenates, at NR concentrations of 10-50 uM, the optical density changes were about .05 to .15 OD units per pH unit.

Supported in part by a grant from the Cleveland Foundation.

285.6 AUTORADIOGRAPHIC STUDIES OF FUNCTIONAL ANATOMY USING 14-C CYANIDE AS A PUTATIVE LABEL FOR CYTOCHROME OXIDASE. R.C. Collins, T. Der* and M.E. Raichle, Depts. of Neurol. and Rad. Sci., Washington Univ. Sch. Med., St. Louis, MO 63110.

Studies using histochemical stains for cytochrome oxidase (cyt-ox) and succinic dehydrogenase suggest that the capacity of oxidative, or mitochondrial metabolism in a neuroanatomic pathway is regulated on a long term basis by functional activity within that pathway (Wong-Riley et al; Durham et al; Marshall et al). We have initiated studies of this relationship using radioactive cyanide as a putative ligand for cytochrome oxidase with the goal of developing a quantitative neuroanatomic assay for this enzyme for autoradiographic studies in animals (14-CN) as well as positron emission tomography (PET) studies in man (11-CN). We gave rats 10 to 30 μCi 14-CN/kg (50 mCi/mmole) iv and assayed arterial blood until sacrifice between 2 and 10 minutes. Final tissue concentrations for grey matter structures (measured autoradiographically, mμCi/gm) varied from 1.2 to 2.0 X white matter. In general, the rank order for structures for binding agreed with our studies of glucose utilization (14-C-Deoxyglucose, DG), blood flow (14-C-iodoantipyrine), and cyt-ox (method of Wong-Riley) as areas of high metabolic rate and high levels of mitochondrial enzyme: inferior colliculus and auditory nuclei, cortex, caudate, thalamus. Binding within certain areas was also consistent - as there was strong labeling of stratum moleculare of hippocampus and dentate gyrus along the perforant path; and in cerebellum labeling was higher in granule cell layer than molecular layer. By contrast some structures with low metabolic rate and low cyt-ox were also heavily labeled, as globus pallidus. This may reflect "nonspecific" cyanide binding with nonhematin (non cyt-ox) iron which is in high concentration in basal ganglia. In the visual system, either acute decreases (enucleation) or increases (strobe light) in functional activity greatly decrease and increase glucose utilization (DG) but not 14-CN binding. Chronic decreases in functional activity - three months following enucleation - results in a decrease in 14-CN binding. These preliminary results suggest that tracer doses of radioactive cyanide may provide a useful way for studying localized functional aspects of mitochrondrial energy metabolism in animals and man.

285.7 INFLUENCE OF URETHANE ON GLUCOSE UTILIZATION IN THE SUPERIOR CERVICAL GANGLION AND IN THE BRAIN. M. Ito*, M. Kadekaro and L. Sokoloff. (SPON: C. Kennedy) Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

Urethane has been widely used in neurophysiological research on autonomic ganglia because it does not block synaptic transmission in anesthetic doses. Although it has been reported that oxygen consumption is decreased in the superior cervical ganglia (SCG) under urethane anesthesia in vitro, neither oxygen consumption nor glucose utilization has ever been determined in vivo under urethane anesthesia.

We have, therefore, employed the 2-[^{14}C]deoxyglucose quantitative autoradiographic method to measure glucose utilization in the SCG and brain of urethane-anesthetized rats (dose, 1 g/kg i.p.). Although the mean blood pressure was significantly lower in anesthetized animals (75±10 mmHg, mean±SEM of 6 rats) compared with controls (133±5 mmHg, n=5), arterial blood pH, pO_2, pCO_2 and hematocrit remained within the normal range. Glucose utilization in the SCG was increased 55% in anesthetized rats (31±1 μmoles/100 g/min, mean±SEM) compared to control conscious animals (20±1 μmoles/100 g/min, n=5). This effect appears to be secondary to humoral factors released during urethane anesthesia because under this condition plasma glucose concentration is significantly elevated, plasma epinephrine levels are increased as much as fourfold, and adrenalectomy prevents the urethane stimulation of glucose utilization in the SCG. In the brain the effect of urethane was markedly different. Of 87 brain structures examined in 4 rats glucose utilization was significantly depressed in 67, particularly cortical structures.

The results of the present study show that urethane depresses glucose utilization in the central nervous system. This effect appears to be overcome in the SCG by humoral factors mediated via the adrenals.

285.8 THE EFFECT OF MODERATE HYPOGLYCEMIA ON MORPHOLOGY IN RAT BRAIN. J. French, D.G. Rawlinson*, W.A. Pulsinelli and T.E. Duffy. Lab of Cerebral Metabolism, Cornell University Medical College, New York, N.Y. 10021.

Specific regions of the cortex, hippocampus and striatum have been shown to be vulnerable to neuronal damage after insulin induced hypoglycemia. However, such studies reflect the morphological damage resulting from severe hypoglycemia associated with a blood glucose concentration below 1.1 mM and an isoelectric EEG. The present study focuses on the morphological changes accompanying mild hypoglycemia (plasma glucose between 1.5 to 2.5 mM) characterized by an EEG consisting of large amplitude, irregular slow waves.

Adult male rats were fasted for 24 hrs and their tail arteries were cannulated under ether anesthesia. Following 3 hrs recovery from anesthesia, the rats were injected intra-arterially with insulin (Iletin U-40; 2.5 to 5.0 units/kg). Monopolar, subdermal leads were used to monitor EEG. The body temperature was maintained at 37°C. Arterial blood was sampled at 15 minute intervals to determine plasma glucose, pH, pO_2 and pCO_2. Hypoglycemia was maintained for 1.0 to 1.5 hrs, at which time half of the rats (n=13) were perfused fixed with formaldehyde or glutaraldehyde solutions and half were injected with glucose (50% in water; 1cc) and allowed to survive for 3 days before being perfused with the same fixatives (n=12). Morphological changes were evaluated from paraffin sections with the light microscope.

Mean systemic blood pressure, pH, pO_2 and pCO_2 were not significantly different from baseline values. All of the rats demonstrated at least 30 minutes of large amplitude, irregular activity on the EEG. There were no clinical or electrographic signs of convulsions. Rats perfused during hypoglycemia showed morphological change in rare neurons in the cortex (11 rats), thalamus (4 rats), hippocampus and striatum (1 rat each). These early changes consisted of cell contraction with increased staining of the cytoplasm and nucleus. Affected neurons in rats perfused after 3 days recovery were even more seldomly observed but the morphology of these late changes was typical of ischemic cell change and occurred in the cortex (5 rats), striatum (2 rats) and hippocampus (1 rat). The cerebellum was histologically normal in both early and late groups.

The data thus demonstrate that even brief, moderate hypoglycemia, of a degree insufficient to produce coma, may irreversibly damage the brain.

285.9

WITHDRAWN

285.10 2-DG UPTAKE IN LESIONED BRAIN: ANESTHESIA PROVIDES A METABOLIC CONTRAST TECHNIQUE. Kirk A. Frey and Bernard W. Agranoff, Neuroscience Lab, University of Michigan, Ann Arbor, MI 48109.

Previous studies from this laboratory have demonstrated a number of alterations in 2-deoxy-D-glucose (2-DG) incorporation into rat brain regions ipsilateral to striatal ibotenic acid lesions. The present work is directed towards determination of the neurochemical and physiological processes underlying the decreased uptake of 2-DG within the lesioned striatum. Ablations were performed by injection of 20 μg of ibotenic acid in 1.0 μl of phosphate-buffered saline (pH 7.4) into the head of the caudate through a 30 gauge cannula. One week following the lesion, regional cerebral glucose metabolism (CMR_{glu}) was examined with [^{14}C]2-DG (Sokoloff et al., J. Neurochem. 28:897, 1977) and was compared with [^{14}C]glucose uptake (Hawkins et al., Stroke 10:690, 1979). Cerebral blood flow was measured using [^{14}C]-iodoantipyrine. CMR_{glu} in the lesioned striatum was decreased by 20% compared to the contralateral (control) side, while blood flow was increased. Animals studied with either [1-^{14}C] or [6-^{14}C]glucose showed an even greater decrease in labeling of the lesion (50% of control) than that observed with 2-DG. The results indicate that there is a luxury perfusion of the striatum one week following ibotenic acid lesion. The difference observed between uptake of 2-DG and glucose suggests that the lumped constant for 2-DG may be altered in brain tissue when the normal coupling between metabolism and blood flow is disturbed. For example, an increase in the ratio of the distribution volume of 2-DG to that of glucose in the lesioned striatum could account for the experimental observations. Alternatively, the relative decrease in labeling observed with [^{14}C]glucose may be secondary to anaerobic metabolism and loss of radioactive lactate from brain.

Uptake of 2-DG was further studied in lesioned animals under barbiturate or ether anesthesia. The lesion-to-control ratio of striatal labeling under these conditions was paradoxically reversed, with the lesioned striatum 20% greater than the control side. Thus, pharmacological suppression of neuronal activity may enhance identification of brain regions where an uncoupling of cerebral blood flow and metabolism has occurred. The use of positron-labeled 2-DG analogs (e.g., [^{18}F]2-FDG or [^{11}C]2-DG) with anesthesia constitutes a contrast method for localization of recent infarction in human brain by positron emission tomography. (Supported by NIH Grant NS 15655. KAF is a trainee of NIH Grant 1 T32 GM07863.)

285.11 NOMOGRAM FOR 2-DEOXYGLUCOSE LUMPED CONSTANT FOR RAT BRAIN REGIONS. W.M. Pardridge. Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

The measurement of rates of regional glucose utilization in brain with 2-deoxyglucose requires the use of a correction factor or lumped constant (LC). Although it has been generally assumed that the LC is constant for a given species, recent studies of the stability of the LC in pathologic states have shown that the LC decreases when the brain/plasma glucose ratio is high (e.g., hyperglycemia) and that the LC increases when the brain/plasma glucose ratio is low (e.g., hypoglycemia, seizures). The variability of the LC in pathologic states is a predictable phenomenon, and is derived from the fact that 2-deoxyglucose transport through the blood-brain barrier (BBB) is about 40% faster than is glucose transport. The relative volumes of distribution in brain of the two sugars differ and thus the LC varies, depending on the existing brain and plasma glucose concentrations. However, the fluctuations in LC with changing brain and plasma glucose can be depicted by a nomogram if seven constants are known. These constants are the K_m, V_{max}, and K_D of glucose and 2-deoxyglucose transport through the BBB and the 2-deoxyglucose phosphorylation coefficient, and these values have been recently published for the cerebral hemisphere and six brain regions (fronto-parietal cortex, hippocampus, olfactory bulb, colliculi, caudate-putamen, and thalamus) (J. Neurochem. 38, 560, 1982). In addition, nomograms have been published for the hemisphere and cortex (J. Cereb. Blood Flow Metabol. 2, 197, 1982). The nomogram provides the LC (z-axis) for any given pair of regional brain (x-axis) or plasma (y-axis) concentration of glucose. The results of the present studies show that the nomograms for the other five regions are not significantly different from the previously published nomogram for the fronto-parietal cortex. Therefore, the latter may be tentatively used as a nomogram representative of all six brain regions studied thus far. Conclusions: The LC may vary more than 3-fold in pathologic states wherein marked changes in glucose distribution in brain occur. Failure to correct for changes in the LC may lead to illusory estimates of local glucose consumption using the 2-deoxyglucose technique. However, measurement of glucose concentrations in plasma and in brain regions and the use of the 2-deoxyglucose nomogram provides a method for approximating changes in the LC in pathologic states.

285.12 ELECTROPHYSIOLOGICAL CONDITIONS DETERMINING INCREASED GLUCOSE METABOLISM MEASURED BY (^{14}C) DEOXYGLUCOSE AUTORADIOGRAPHY, Barry S. Layton, Samuel David* and Leo P. Renaud. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, and Division of Neurology, Montreal General Hospital.

We have used the anatomically well-defined system of the amygdala - stria terminalis (ST) - and bed nucleus of the stria terminalis (BST) to investigate some of the electrophysiological conditions necessary for functional mapping with ^{14}C-2-deoxyglucose (2-DG) autoradiography. Bipolar stimulating electrodes were implanted unilaterally in basal or cortical amygdaloid nuclei in pentobarbitol anesthetized male Sprague-Dawley rats. Three types of experiments were defined according to the conditions of stimulation: 1) continuous stimulation at 2, 7.5 or 20 Hz beginning after I.V. injection of 2-DG; 2) pulsed stimulation at 20 Hz for 1 sec every 10 seconds after 2-DG injection; 3) pretreatment stimulation at 20 Hz continuously for 1 hour followed by injection of 2-DG and stimulation at 2 Hz or no stimulation at all. The duration of all experiments was 45 minutes after administration of the 2-DG. D.C. current was then passed through the electrodes to mark the location of the tips and the brains processed according to Sokoloff et al. (J. Neurochem. 28, 897, 1977). The ST and BST ipsi- and contralateral to the stimulated amygdala were identified in autoradiographs by reference to adjacent Nissl-stained sections and their optical densities determined.

Autoradiographs from animals in which 2-DG was administered immediately upon beginning continuous stimulation at 7.5 and 20 Hz or pulsed stimulation at 20 Hz exhibited striking side to side differences in the optical density of both the ST and BST. Similar results occurred when pretreatment at 20 Hz was followed by continuous stimulation at 2 Hz. Neither continuous stimulation at 2 Hz nor pretreatment alone resulted in detectable increased accumulation of labelled metabolite in either structure.

These results suggest that the frequency and pattern of stimulation as well as the history of activation immediately prior to the injection of 2-DG determine the amount of glucose metabolism and therefore the optical density of CNS structures visualized in autoradiographs.

285.13 AN INEXPENSIVE MICROCOMPUTER-BASED VIDEO ACQUISITION SYSTEM FOR ANALYZING X-RAY AND HIGH RESOLUTION DEOXYGLUCOSE AUTORADIOGRAMS. Doron Lancet* and Ami Isseroff. Depts. of Membrane Res. and Isotope Res., The Weizmann Inst. of Sci., Rehovot 76100, Israel.

The processing of image data is important for many neurobiological applications, such as neuroanatomical reconstructions, mapping of neural metabolic activity and localization of neurotransmitter receptors. Quantitative and reproducible treatment of such data requires computer assisted analysis, preferably aided by video acquisition of complete image frames.

Using commercially available components, we have constructed an inexpensive, general purpose image analysis system, which is being utilized in our laboratories for processing X-ray film and high resolution 2-deoxyglucose (2DG) autoradiograms. Whole frames are entered through a television camera from a light microscope or by direct viewing. A specialized interface (conforming to the S-100 bus standard) with image refresh memory and color graphics capabilities, digitizes the video frame in 1/30 sec at a resolution of 512X512 picture elements and 256 gray levels. The system is controlled by a microcomputer with a Z-80A processing unit equipped with standard peripherals and magnetic disk storage. Image oriented user interaction is provided by a light pen, a joystick and an X-Y digitizer tablet.

The capabilities of this system are similar to those of more expensive minicomputer-based hardware configurations currently in use. Optical densities may be read off the X-ray film autoradiograms and transformed into false color representation of gray levels. Images can be treated by contrast enhancement algorithms or averaged for noise reduction. Three dimensional reconstructions or various cross sections of labelled structures may be displayed. Image subtraction may be performed for analysing double labelling experiments.

We have recently applied a modified 2DG autoradiographic method to the mapping of metabolic activity with cellular resolution in whole regions of the nervous system of behaving vertebrates (Lancet, D., Greer, C.A., Kauer, J.S. and Shepherd, G.M., Proc. Nat. Acad. Sci. (USA) 79:670, 1982). The image analysis system described here makes it possible to record, store and analyse the large quantities of data produced by this newly developed technique. Uptake of 2DG in different cell populations or regions of synaptic terminals may be measured and statistically analysed using silver grain counting, activity distribution histograms and stereometry. This affordable yet sophisticated system should greatly extend the application of 2DG autoradiography in the study of neuronal information processing at the regional and cellular levels.

285.14 METABOLIC MAPPING OF NIGRAL CONNECTIONS WITH THE 2-[^{14}C]DEOXYGLUCOSE METHOD. A. Pert§, H.D. Everist§, H.H. Holcomb§, M. Kadekaro§§, P.M. Gross§§, and L. Sokoloff§§. (SPON: D. Van Kammen). Biological Psychiatry Branch§ and Laboratory of Cerebral Metabolism§§, National Institute of Mental Health, Bethesda, Maryland 20205.

Electrical stimulation of inputs to neuronal structures has been shown to increase their rate of glucose utilization. By stimulating the substantia nigra and medial forebrain bundle, we have mapped functional neuronal circuits with the 2-[^{14}C]deoxyglucose method. In male Sprague-Dawley rats with chronically implanted electrodes, unilateral stimulation of the substantia nigra (biphasic pulses of 750-1000 μA, 100 Hz, 50 μs) evoked characteristic behavioral responses such as ipsilateral shoulder and head rotation and forepaw planting that were synchronous with the stimulation. In contrast to sham controls the metabolic pattern in stimulated animals displayed marked and selective increases. Glucose utilization was elevated in the ipsilateral subthalamic nucleus by 38% (contralateral: 68±7 μmoles/100 g/min; ipsilateral: 94±11, means ± SE, n=4) and in the entopeduncular nucleus by 85% (contralateral: 55±6; ipsilateral: 102±4, n=4). An increase in glucose utilization also occurred in the ipsilateral globus pallidus. To evaluate the relative contributions of orthodromic and/or antidromic activation of afferent and efferent connections of the substantia nigra, we examined the metabolic patterns resulting from unilateral stimulation of the medial forebrain bundle. In contrast with the effects of nigral stimulation, no increases in glucose metabolism were found in the ipsilateral subthalamic, entopeduncular or pallidal nuclei. A marked activation of glucose utilization was observed, however, in the ipsilateral zona compacta of the substantia nigra and along the medial forebrain bundle up to its most rostral projections. These results indicate that the regional increases in metabolism during nigral stimulation resulted from both orthodromic and antidromic activation of nigral connections. Lesions and pharmacological manipulations should reveal the relative importance of these two mechanisms.

285.15 NALOXONE PRETREATMENT MODIFIES THE CEREBRAL METABOLIC EFFECTS OF γ-HYDROXYBUTYRATE IN RATS. G. Crosby, M. Ito*, E. Kaufman*, T. Nelson*, L. Sokoloff. Lab. of Cerebral Metabolism, NIMH, Bethesda MD 20205, and Dept. Anesthesia, Univ. of Penna, Phila. PA 19104.

γ-Hydroxybutyrate (GHB) is normally present in brain (Roth, R.H., Biochem. Pharmacol., 19:1087, 1970), but when administered in pharmacologic doses it produces anesthesia characterized by profound reductions in cerebral metabolism (Wolfson, L.I., J. Neurochem., 29:777, 1977). A recent report (Snead, O.C., III, Neurology, 30:832, 1980) that some of GHB's effects are naloxone-reversible suggests an opiate-mediated mechanism of action which thus far has not been conclusively demonstrated for other non-narcotic anesthetics. The present experiment was designed to test whether naloxone antagonizes the cerebral metabolic depression by GHB. In order to do so, local cerebral glucose utilization (LCGU) was measured with the quantitative 2-[^{14}C]deoxyglucose method (Sokoloff, L., J. Neurochem., 28:897, 1977) in 6 rats treated with naloxone, 10 mg/kg IP, followed 15 min later by γ-butyrolactone (GBL) 200mg/kg IP; and in six other rats which received GBL alone. Pharmacologically inactive itself, GBL is rapidly converted to GHB by a lactonase present in plasma and liver (Roth, R.H., Biochem. Pharmacol., 16:596, 1967). LCGU measurements started 5 min after GBL administration. The autoradiographs produced by the method were analyzed with a manual densitometer. Statistical comparisons were made with a group t-test.

GBL produced a rapid 1.0 to 1.5°C temperature reduction; naloxone pretreatment reduced the magnitude of the decline. The glucose utilization of structures in naloxone-pretreated rats was always higher than in animals given GBL alone. The increase was statistically significant in only 12 of the 38 structures examined; in these LCGU increased 20 to 40%. Structures affected include 3 cerebellar regions, ventral thalamic and subthalamic nuclei, substantia nigra, inferior colliculus, periaqueductal and pontine gray, dorsal raphe, vestibular nuclei, and the internal capsule. The present data show only partial antagonism by naloxone of the cerebral metabolic depression produced by GHB. Because of the selective nature of the effect, temperature differences between groups cannot explain the results. Unfortunately, little relationship is apparent between the regional distribution of either endogenous GHB (Rumigny, J.F., J. Neurochem., 36:1433, 1981) or opiate receptors (Atweh, S.F., Brain Res., 129:1, 1977) and those structures showing naloxone-reversible metabolic depression. Our data therefore support the ability of naloxone to at least partially reverse the central effects of GHB.

285.16 REGIONAL INCORPORATION OF [14C]-PALMITIC ACID INTO THE BRAINS OF ANESTHESIZED RATS. A.S. Kimes and S.I. Rapoport. Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, Maryland 21224.

Previously we reported (Kimes and Rapoport, Neurosci. Abst. 7:911, 1981) that the regional brain incorporation of i.v. injected [14C]-palmitate correlates well with local cerebral glucose utilization (LCGU) in the conscious, resting rat as determined by the method of Sokoloff et al. (J. Neurochem. 28:897-916, 1977). Since LCGU has been correlated with functional activity in many experimental conditions (i.e. anesthesia, enucleation, stimulation), we compared the regional incorporation of [14C]-palmitate into the brain of anesthesized rats with that of conscious rats. If palmitate incorporation is related to functional activity, altering the functional state of the animals should change palmitate incorporation.

All rats were anesthesized for catheter placement with sodium pentobarbital (50 mg/kg). One group of rats was maintained under pentobarbital anesthesia (anesthesized group) and the other (control group) was allowed 4.5 h to recover from the anesthesia. Both groups were injected with 450 μCi/kg [14C]-palmitate and timed arterial blood samples were withdrawn via the catheters. The animals were decapitated at 4 h after injection. Their brains were rapidly frozen, cut into 20 μm sections, and placed against x-ray film with standards for 2 months. Regional optical densities in the autoradiographs were converted to dpm/mg brain using calibrated standards.

Brains of anesthesized animals contained about twice the radioactivity found in control brains in all regions. Values for anesthesized brains were 105 dpm/mg tissue (white matter) up to 300 dpm/mg (inferior colliculus), while the range for the control brains was 70 dpm/mg to 165 dpm/mg tissue. However, the radioactivity in the plasma of the anesthesized animals was also much higher, probably due to a lower overall metabolic rate in the anesthesized state. Normalizing both the experimental and control regional brain values by dividing by the area under the plasma curve corresponding to palmitate, gave different results. Regional values in normalized anesthesized brains were 25-50% lower than the regional control brain values. These decreases are similar to those found by Sokoloff (Neurosci. Res. Prog. Bull., 1976 pp. 454-520) using 2-deoxyglucose to measure LCGU in anesthesized and conscious rats. These data indicate that [14C]-palmitate incorporation is related to the functional level of the brain.

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285.17 RESPONSES OF CEREBRAL UNIT ACTIVITY TO GRADUAL DECLINING OXYGEN TENSION R. M. Martin and J. H. Halsey. Dept. of Neurology, University of Alabama in Birmingham, Birmingham, AL 35294.

There is currently in debate the question of selective vulnerability of neurons to hypoxia and/or ischemia. Namely, are certain neurons more capable of withstanding these insults due to their proximity to the surrounding capillaries and thus a higher oxygen content or through a lower metabolic or functional demand? The Krogh cylinder model of capillary/tissue geometry suggests regions of very low PO_2 for cells supplied near the venous end of the capillary. Possibly, these cells would suffer the first effects of hypoxia through the immediate loss of oxygen availability. With our current ability to measure action potentials simultaneously with PO_2 from the oxygen microelectrode, we have attempted to investigate this question. Recording from cortical and hippocampal neurons were performed in mechanically ventilated gerbils exposed to a falling inspired PO_2 (25% O_2 to 0% O_2 in 90 sec). Parameters noted were normal resting PO_2, PO_2 at which unit activity is altered, PO_2 at which activity ceased, and the unit's ability to recover. Preliminary indications show there is no clear relationship between resting PO_2 and the PO_2 at which activity fails, for some cells at high PO_2 failed only after a PO_2 of 0 was attained and other cells at low PO_2 failed at PO_2's above 0. It was interesting to note that of 13 cells initially recorded, only one displayed any sign of recovery within a 15 minute post-hypoxia monitoring period. This one cell possibly may indicate a more hypoxic resistant variety of neuron within the CNS. To address the question of capillary proximity, we have begun marking the measuring site by iron deposition (Nair et al., J. Appl. Physiol. 49:916, 1980) for subsequent microscopic analysis of capillary distribution. NIH support.

285.18 TEMPORAL PROFILE OF REGIONAL HIGH-ENERGY METABOLITES IN RAT BRAIN AFTER TRANSIENT FOREBRAIN ISCHEMIA. W.A. Pulsinelli, T.E. Duffy, Dept. Neurol., Cornell Un. Med. College. New York, N.Y. 10021

We have demonstrated in rats exposed to 30 min of forebrain ischemia that the onset of morphological damage to dorsal-lateral striatal neurons occurs within 3-6 hr of cerebral reperfusion while the onset of damage to the CA1 pyramidal neurons in hippocampus is delayed for 24 hr (Ann Neurol 11:491, 1982). The number of damaged neurons becomes maximal in the striatum at 24 hr and in the CA1 zone at 72 hr. Blood flow rises above control values and glucose metabolism falls in both regions as maximal tissue injury is attained (Ann Neurol 11:499, 1982). We report here measurements of regional high-energy metabolite levels during and at serial intervals after transient forebrain ischemia.

Adult rats were surgically prepared under general anesthesia for 4-vessel occlusion by electrocauterizing the vertebral arteries and 24 hr later temporarily occluding the carotid arteries for 30 min. At 1, 6, 24, 48 and 72 hr after cerebral reperfusion the rats were paralyzed with curare and mechanically ventilated. The skin and subcutaneous tissues overlying the skull were anesthetized with xylocaine and the rat brains were frozen, in situ, by pouring liquid N_2 into a funnel fixed to the skull. Phosphocreatine (PCr), ATP and lactate were measured in samples of paramedian neocortex, dorsal-lateral striatum and the CA1 zone of hippocampus using enzyme-fluorometric procedures.

	DORSAL-LATERAL STRIATUM			CA1 HIPPOCAMPUS		
	ATP	PCr	Lact.	ATP	PCr	Lact.
Controls	2.74	4.58	0.90	2.25	5.17	1.24
30 min Isch.	.09†	.06†	10.08†	.06†	.06†	12.73†
Reperf. 1 hr.	1.99†	5.45*	1.14	1.69†	6.10*	1.86
6 hr.	2.32	4.71	1.71	1.86*	5.88	1.36
24 hr.	1.48†	3.29†	5.39†	2.42	5.72	1.45
48 hr.	1.50†	3.15†	5.10†	2.11	5.11	2.99*
72 hr.	1.87†	3.83	2.26	1.55†	3.95†	3.02†

Values = means (mmol/Kg); N=4-5 animals/group; *$p < .05$, †$p < .01$

In the reperfusion period ATP, PCr and lactate levels recovered to control (hippocampus, 24 hr) or near-control (striatum, 6 hr) values and then showed a secondary failure of mitochondrial respiration. In the paramedian neocortex, a region with no morphological damage, ATP, PCr and lactate values returned to control levels at 24 hr and remained normal at 48 hr (values not shown). The late changes in ATP, PCr and lactate observed in striatum and hippocampus (table) coincided temporally with the delayed changes in morphological damage, blood flow and glucose metabolism previously described. The results indicate that cells in these regions regain normal or near-normal mitochondrial function and are viable, in terms of energy production, for many hours before unknown mechanisms cause irreversible damage.

285.19 BRAIN PROTEIN SYNTHESIS IN HIBERNATING GROUND SQUIRRELS. E. L. Bennett, M. H. Alberti*, and M. R. Rosenzweig. Melvin Calvin Laboratory and Department of Psychology, University of Calif. Berkeley, CA 94720.

The relative rate of protein synthesis of the hibernating Belding's ground squirrel (Spermophilus beldingi) has been compared with that of the awake ground squirrel based upon the incorporation of L-U-[^{14}C]-valine into the TCA-insoluble (protein) fraction of brain. In the awake squirrel, the uptake into brain is approximately linear during the first hour after subcutaneous administration of 30 μCi [^{14}C]-valine. The specific activity of brain was 45 nCi/g at 1 hr, and approximately 50% was converted into the TCA insoluble (protein) fraction at this time. In the hibernating squirrel, the uptake was approximately 100 nCi/g at 1 day, and little change was noted over the next week. However, in the hibernating squirrel the conversion into TCA insoluble material was extremely slow, attaining a maximum value of 1% after 8 days of incorporation. One measure which may be derived to compare the relative rates of incorporation is TCA insoluble radioactivity over total radioactive uptake/hr. This value is approximately 50% in the awake squirrel and 0.008% in the hibernating squirrel. We conclude that the rate of protein synthesis in the brain of hibernating squirrels is on the order of 0.015% of that of the awake squirrel. Similar estimates for the relative rates in liver yield a value of approximately 0.08%. Furthermore, the rate of protein synthesis in brain during hibernation appears to be much lower than the rate of basal metabolism, which is estimated to be about 3% of the waking value. Relative rates of protein synthesis in various brain regions are now being investigated by autoradiographic methods.

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285.20 The Binding and Distribution of Zinc in the Hippocampus and Cerebellum. S.M.Sato*, J.M.Frazier* and A.M.Goldberg. Dept. of Environmental Health Sciences, Johns Hopkins Univ., Baltimore, MD 21205

Zinc is generally regarded as being uniquely concentrated in the hippocampus of all species studied. Histochemical studies have demonstrated a concentrated, cytoplasmic pool of zinc which is localized in the mossy fiber boutons of the hippocampus. Quantitative analyses of zinc in the brain support the histochemical observations and demonstrate higher concentrations of zinc in the hippocampus as compared with other brain regions. However, the mechanism by which zinc is concentrated and sequestered has not been elucidated. Three mechanisms may explain regional differences in brain zinc: 1)Unique zinc binding site/s 2)Increased zinc binding capacity, or 3)A specific uptake system for zinc. We have tested the hypothesis that zinc may be sequestered by means of a unique cytoplasmic binding species localized in the hippocampus. We compared the hippocampus with the cerebellum. The cerebellum was chosen as a control region since it does not appear to sequester and concentrate zinc and it has been reported to contain lower zinc levels by both quantitative and qualitative studies.

Initially, we established a quantitative difference in zinc content between the hippocampus and the cerebellum which correlated well with previously determined heavy metal localization patterns in the brain. The cerebellum was found to contain 30% less zinc as compared with the hippocampus, (9.25 ± .59 ug zinc/g wet wt. tissue in the cerebellum compared with 12.60 ± .85 ug zinc/g wet wt. tissue in the hippocampus, X + S.D., n=6). Using a modified subcellular fractionation method, we observed that this quantitative difference in the amount of zinc between the hippocampus and cerebellum is largely reflected in cytosolic zinc levels--the cerebellum being 40% lower than the hippocampus,(3.19 ± .54 ug zinc/g wet wt. tissue in the cerebellum as compared with 5.26 ± .86 ug zinc/g wet wt tissue in the hippocampus).

Three zinc-binding species in the cytosolic fraction from both the hippocampus and the cerebellum have been identified using column chromatography (Ultrogel AcA 34).There appears to be no qualitative difference in the zinc binding species localized in the hippocampus and the cerebellum suggesting the difference in zinc levels is probably not attributed to a unique binding site.

Since the levels of zinc are different in the hippocampus and the cerebellum and there does not appear to be a unique binding species in the hippocampus, another mechanism, e.g. capacity or uptake, might account for the differences in zinc levels. We are currently investigating the zinc-binding capacity of the hippocampus and the cerebellum to determine if quantitative differences in the amount of zinc-binding species in the two regions might explain the observation.

285.21 RAISED INTRAVENTRICULAR PRESSURE SELECTIVELY IMPAIRS CEREBRAL BLOOD FLOW IN WHITE MATTER. G.A. Rosenberg* and W.T. Kyner* (SPON: L.D. Partridge). Depts. of Neurology, Physiology, and Mathematics, Univ. of New Mexico, and Veterans Admin. Med. Ctr., Albuquerque, NM 87131

Patients with chronic communicating hydrocephalus have reduced cerebral blood flow (CBF). In the early stages of hydrocephalus the CSF pressure is elevated. Therefore, we studied the effect of increased intraventricular pressure on CBF in cats. CSF pressure was raised during ventriculocisternal (VC) perfusions to 20 cm H_2O. CBF was measured in 10 animals with ^{123}I-iodoantipyrine at the end of 4-hr perfusion. Three animals had pressures of 20 cm H_2O, 4 were hypocapnic, and 3 served as controls (-5 cm H_2O pressure). We found that animals with raised pressures had a selective reduction in CBF in periventricular white matter from a control value of 0.19±0.02 (SEM) to 0.10±0.02 ml/min/gm ($p<0.001$; Student t-test). CBF to caudate and cortex was unaffected whereas hypocapnia reduced CBF in all three areas. Additional animals underwent VC perfusions at -5 or 20 cm H_2O pressure with CSF containing horseradish peroxidase (HRP). The HRP reaction product was found in periventricular white matter at the higher pressure with transependymal bulk flow of CSF observed in perivascular spaces and fiber tracts. The impairment in CBF occurred at sites of transependymal bulk flow. Since normally brain interstitial fluid is cleared into the ventricle during low pressure VC perfusion, the impaired CBF may have been due to alterations in the perivascular fluid environment. Another possible explanation is compression of blood vessels at the raised pressure. Reversible impairment in gait seen with hydrocephalus may be related to a selective disturbance of CBF in periventricular white matter.

286.1 EXPERIMENTAL NEURORETINAL DESTRUCTION BY ACTIVATED MACROPHAGES. M. del Cerro[1,2], A.A. Monjan[3]*, D.A. Grover[2]*, J. Dematteo[1]* and M.F. Kritzer[1]* Center for Brain Research[1] and Department of Ophthalmology[2], University of Rochester, Rochester, NY, 14642, and Department of Epidemiology[3], Johns Hopkins University, Baltimore, MD.

The retinitis induced by lymphocytic choriomeningitis virus (LCMV) inoculation of neonatal rats has been studied by combined clinical, electrophysiological, and histomorphological methods. The infection causes two consecutive forms of retinitis. The first is an acute, virus-specific, immunopathogenic destruction of infected cells in the neural retina consequent with virus clearance. This stage, which lasts approximately 8 weeks, is characterized by the presence of extensive cell dysplasia and subretinal hemorrhages. The end result is a retina deprived of inner and outer segments over substantial portions of its surface. The second disease is a chronic inflammatory state of indefinite duration. It is characterized at all times by an extensive invasion of the retina by activated macrophages, which leads relentlessly to obliteration of the neural retina. This persistent retinitis is presumed to be triggered by the release of sequestered retinal antigens at the site of the acute lesion. The LCMV ocular disease offers a safe, economical and highly reliable animal model for the study of the pathogenesis and treatment of immune retinitis.

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286.2 FLUORESCENCE POLARIZATION ANALYSIS OF SYNAPTOSOMAL MEMBRANES FROM CATS WITH GM_1 GANGLIOSIDOSIS. P.A. WOOD*, M.R. McBRIDE*, H.J. BAKER*, S.T. CHRISTIAN. [SPON: A. MAIER]. DEPTS. OF COMPARATIVE MEDICINE AND PSYCHIATRY, UNIVERSITY OF ALABAMA IN BIRMINGHAM, AL 35294.

The gangliosidoses are a group of inherited metabolic diseases that cause progressive neurologic dysfunction of unknown pathogenesis. Previous studies on cats with GM_1 gangliosidosis provided morphologic and functional evidence suggesting that neuronal plasma membrane may have altered structure, perhaps due to excess GM_1 ganglioside content. We tested this hypothesis by evaluating fluorescence polarization characteristics of the fluorescent probe 1-acyl-2-(N-4-nitrobenzo-2-oxa-1, 3-diazole)-aminocaproyl phosphatidylcholine (NBD-PC) incorporated into synaptosomal membranes from normal cats and cats in advanced stages of GM_1 gangliosidosis. The results showed significantly reduced fluidity of synaptosomal membranes from cats with GM_1 gangliosidosis which is consistent with presence of excess GM_1 ganglioside. We are studying currently the Na+-K+ATPase, Ca++ ATPase and Ca++ efflux kinetics of these synapsomal membranes.

286.3 THE THREE-DIMENSIONAL DISTRIBUTION OF NERVE DAMAGE FROM MICROSPHERE EMBOLIZATION OF NERVE CAPILLARIES, H. Nukada* and P.J.Dyck, (SPON: T. Yanagihara). Mayo Clinic, Rochester, MN 55905.

Endoneurial capillaries play important roles in nerve nutrition, waste removal and maintainance of microenvironment homeostasis. In lead, diabetic and possibly other neuropathies, the nerve microenvironment is altered, suggesting capillary dysfunction. Intra-arterial injection of arachidonic acid may be associated with ischemic nerve damage (Parry & Brown, 1982). Intravascular platelet aggragation & vasoconstriction are thought to lead to occlusion of pre-capillary arterioles & large capillaries. Whether the three-dimensional pattern of fiber degeneration, is the same or different than from widespread arteriolar or arterial occlusive disease is unclear. We injected polystyrene microspheres (15±3 μm, OD, 3M, St. Paul, MN, U.S.A.) intra-arterially to produce capillary occlusion of lower limb nerves of adult rat. Initially, radiolabelled (Cr-51) microspheres were injected into external iliac, internal iliac & superior gluteal arteries. At different time intervals after injection, consecutive segments of desheathed sciatic nerve & its branches from proximal to distal, were shown to contain radioactivity which had not decreased by 6 days. The distribution of radioactivity along the nerves were somewhat different among arteries injected. Irrespective of the vessel injected, the highest concentrations of radioactivity (counts per endoneurial weight) were at the mid-thigh level corresponding to the distal sciatic nerve & the proximal tibial, peroneal & sural nerves. In subsequent studies we injected 2 million non-radioactive microspheres into each of the arteries without their permanent ligation. Foot & leg weakness & corresponding sensory loss were invariable. A week later the sciatic nerve & branches & the regional arteries were fixed in situ & consecutive tissue blocks along the length of the nerve & vessels were embedded into epoxy for transverse sections. Microspheres were observed occluding some endoneurial capillaries but no regional arteries or epineurial arterioles. No fiber pathology was found for the upper sciatic nerve. At a somewhat variable level, central fascicular fiber degeneration & loss began at mid-thigh level & tended to become more diffuse & severe distally. The three-dimensional pattern of fiber degeneration appears to be similar to the findings in human necrotizing angiopathy (Dyck, Conn & Okazaki, 1972) from widespread segmental occlusion of arterioles, & to experimental occlusion of large arteries (Korthals & Wisniewski, 1975; Hess et al, 1979). A difference in the pattern of ischemic nerve damage from occlusion of vessels of different size has thus far not been observed. Because capillaries can be selectively occluded resulting in ischemic nerve damage, this microsphere embolization model should be a good one for the study of human microangiopathic neuropathies.

286.4 LYSOSOMES AND METABOLISM OF GLUCOSYLCERAMIDE IN NORMAL AND GLUCOSYL CERAMIDOTIC HUMAN SKIN FIBROBLASTS. M. Saito* and A. Rosenberg* (SPON: J. Trimble). Dept. of Biochem. and Biophys., Loyola Univ. Stritch School of Medicine, Maywood, IL 60153.

Two classes of lysosomes comprising a dense and a light fraction, were separated from both normal and glucosylceramidotic cultured human skin fibroblasts by centrifugation in a colloidal silica density gradient by the method of Rome et.al. (Cell 17, 143. 1979). The activities of several lysosomal glycosidases in the light fraction increased in density-inhibited cultures of both cell types. An increase in the activity of β-galactosidase in the light fraction was also found in serum-deprived cultures. These phenomena might be related to the autophagosome formation in these cultures. Higher activity of β-galactosidase in the light fraction was also detected in cells detached by trypsin and incubated for 1h at 37°C compared with cells kept at 4°C after trypsin treatment. β-Galactosidase and β-glucosidase in the light fraction were more stable than those in the dense fraction. The proportion of the light fraction found in density-inhibited cultures of infantile Gaucher's disease fibroblasts was higher than that found in density-inhibited cultures of adult Gaucher's disease. No difference was observed between normal and Gaucher's disease fibroblasts with regard to the density of lysosomes although β-glucosidase activities were greatly reduced in both dense and light fractions in Gaucher's cells. When Gaucher's cells were cultured in media containing glucosyl [^{3}H] ceramide for 7 days, no accumulation of labeled materials was observed in lysosomal fractions and the labeled materials were found in the plasma membrane fraction.

Analysis of the labeled materials revealed that a significant amount of radioactivity (about 10%of the radioactivity found in the glycosylceramide fraction) was in the GM_3 and GM_2 fractions. We also analyzed glycosphingolipids of non-labeled cells and could not detect a significant increase in the amount of glycosylceramide in Gaucher's cells compared with that in normal fibroblasts even after long cultivation. These results might indicate that glucosylceramide generated in the catabolic pathway can be used for the synthesis of polyglycosylceramides as suggested previously (Barton & Rosenberg, J. Biol. Chem. 250, 3966, 1974).

286.5 ENDOTHELIAL MICROVILLI PRODUCED BY DIFFUSE CEREBRAL ISCHEMIA. W.D. Dietrich, R. Busto*, and M.D. Ginsberg*. Cerebral Vascular Disease Research Center, Departments of Neurology and Anatomy, University of Miami School of Medicine, Miami FL 33101.

It has been hypothesized that microvascular alterations may be responsible for focal impairment of cerebral reperfusion following periods of ischemia. In order to assess this possibility, we subjected 25 male Wistar rats to a period of global forebrain ischemia of 7 - 30 minute's duration, by the method of Pulsinelli and Brierley (Stroke 10: 267-272, 1979). In some instances, this was followed by a postischemic recirculation period lasting up to 4 hours. The intraparenchymal vasculature was systematically examined by scanning electron microscopy of vibratome sections; in this manner, large areas of the luminal surface of vessels could be surveyed. With this approach, we were able to demonstrate consistent endothelial alterations in areas of brain parenchyma shown by transmission electron microscopy to be vulnerable to this ischemic insult (neocortex, striatum, hippocampus). The salient finding was the appearance of multiple microvilli on the luminal surface of vascular endothelium. The surface projections were relatively rare in sham-operated controls but were documented by morphometric procedures to increase progressively in frequency with the increasing duration of the ischemic insult. Transmission electron microscopy performed on adjacent serial vibratome sections confirmed the presence of these vascular alterations, although these cross-sectional views did not readily permit assessment of their density. Following 4 hours of postischemic reperfusion, some endothelial cells appeared to be more severely affected. The endothelial microvilli observed in this study were sufficiently dense and numerous as to support the view that they may constitute a hemodynamic impediment during postischemic recirculation. Thus, the results of this study suggest a link between these observed vascular alterations and the syndrome of post-ischemic hypoperfusion. Although the present data do not allow an inference as to the mechanisms of formation of these endothelial projections, the results form the basis for future avenues of investigation.

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286.6 ENDOTHELIAL CELL CHANGES IN EXPERIMENTAL HYPERTENSION. P.A. Grady and O.R. Blaumanis*. Cerebrovascular Research Center, Department of Neurology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

The relationship between hypertension and cerebral vascular pathophysiology has been studied epidemiologically in the Framingham Study where it was determined that hypertension was the most important risk factor in the incidence of stroke.

We have examined the effect of acute and chronic hypertension on the endothelial lining of intracranial and extracranial arteries. Experimental hypertension was created in mongrel dogs using the one-kidney one-clip Goldblatt technique and in CDF Albino rats using the two-kidney one-clip Goldblatt technique. Blood pressure was monitored using the indirect tail cuff method in rats and forepaw readings in dogs. Animals were perfused from 8 days to six months after the onset of hypertension and major intra- and extracranial arteries were removed and prepared for scanning electron microscopy (SEM).

A number of morphological changes were seen in the endothelial cells lining the blood vessels. After one month of hypertension subtle restructuring was observed on the luminal surfaces of vessels. Such changes included pitted surfaces, strands or endothelial cords, cusps, and occasionally areas of raised nuclei and necrotic endothelial cells. Occasional deposits of fibrin and platelets or platelet and red blood cell thrombi were observed. These changes were not seen in control animals.

In conclusion, it appears that hypertension of a duration as brief as one to 3 months results in endothelial injury and restructuring of the endothelial surfaces of brain vessels. It appears likely that these early changes in combination with other risk factors such as hyperlipidemia may act together to precipitate further cerebral vascular pathology.

286.7 TIME-DEPENDENCE OF, AND EFFECTS OF INHIBITION AND CELLULAR AGING ON, CHLORIDE EFFLUX ACROSS ERYTHROCYTE MEMBRANES IN HUNTINGTON's DISEASE. D. A. Butterfield*, and W. R. Markesbery* (SPON: D. T. Frazier). Departments of Chemistry, Neurology, and Pathology, and Sanders-Brown Research Center on Aging, University of Kentucky, Lexington, KY. 40506.

Previous studies from our laboratory have demonstrated an increased rate of chloride transport across erythrocyte membranes in Huntington's disease, a process regulated at the external side of the major transmembrane protein Band 3 [Bialas et al (1980) Biochem Biophys Res Comm 95: 1895-1900]. A marked effect of time was noted in three Huntington's disease samples that were studied more than four hours after obtaining the blood. In order to study anion transport more closely in Huntington's disease and the apparent time dependence of chloride efflux in this disorder, we have performed several sets of experiments. Chloride transport in Huntington's disease erythrocytes, obtained from patients giving informed consent (approved by the University Human Subjects and Investigations Committee), was found to be extremely sensitive to time with the efflux rate constant decreasing by approximately 30% over a 24 hour period. In contrast, chloride transport in control cells was unaffected by time. Inhibition studies with the specific anion transport blocker 4,4´-diisothiocyanto-2,2´-disulfonic acid stilbene (DIDS) demonstrated that the same degree of inhibition of chloride transport could be achieved at a much lower concentration of DIDS in Huntington's disease than in controls. Comparison of chloride efflux in fractions enriched in young and old erythrocytes, respectively obtained by density centrifugation of fresh blood, demonstrated that only in the young fraction of cells was chloride efflux diminished with time in Huntington's disease ($p < 0.02$). Chloride transport in in-vivo aged Huntington's cells and in both young and old control cells were essentially not dependent on time. These results will be discussed in terms of proposed molecular mechanisms for neuronal loss in this disorder. The alterations in chloride efflux in erythrocytes are consistent with a cell-surface membrane defect involving a protein in Huntington's disease. Supported in part by NIH grants NS-13791, AG-02759, AG-0084.

286.8 L-HOMOCYSTEIC ACID AS AN ALTERNATIVE MODEL FOR STUDYING L-GLUTAMATE INDUCED CELLULAR DEGENERATION IN HUNTINGTON'S DISEASE FIBROBLASTS. P.N. Gray and P.C. May. Dept. Biochemistry and Molecular Biology, Univ. of Oklahoma HSC, Oklahoma City, OK 73190.

L-Glutamate (Glu) treatment of skin fibroblasts in culture from persons with Huntington's disease (HD) can be used as a model system for studying HD (Gray, et al, BBRC 95:707, 1980). Glu induces cellular degeneration with HD cells being more sensitive than the controls. No natural animal model of HD has been found but a non-genetic partial phenocopy can be generated by direct injection of Glu or kainic acid into adult rat striata (McGeer and McGeer, Nature 263:517, 1976; Coyle and Schwarcz, Nature 263:244, 1976). The lesioning capacity of the kainate is blocked if the cortical tracts providing glutamatergic input to the striatum are severed, suggesting an interaction between endogenous glutamate and exogenous kainate (Biziere and Coyle, J. Neurosci Res 4:383, 1979). These results support the hypothesis of Glu induced neuronal degeneration in HD. In order to develop an alternative system in which a non-metabolizable glutamate analog is used to produce differential sensitivity between HD and control cells both cell types were exposed to several neurotoxins (e.g., kainic acid, N-methyl D, L-aspartic acid, aspartic acid, cysteic acid, L-homocysteic acid (HCA) and D-homocysteic acid) at concentrations up to 30mM. Glu induced death was characterized by plasma membrane blebbing and cellular fragmentation with an LD_{50} of 20.5mM. Similar degeneration was observed in control cultures exposed to Glu concentrations $\geq$ 35mM. Only L-HCA acid, the sulfonic acid analog of Glu, was toxic at 30mM to both HD and control cells (11.4% and 32.3% viable, respectively). The other compounds did not induce cell death. The degeneration accompanying L-HCA induced cell death was morphologically indistinguishable from the Glu induced death and suggests a similar toxic mechanism. The attenuated response of both cell types to D-HCA and D-Glu indicated a stereoselectivity for at least one step of the toxic mechanism. L-HCA is more cytotoxic than L-Glu. The LD_{50} for L-HCA was 3.5mM, only 14.3% of the L-Glu LD_{50}. The LD_{50} of L-HCA for the control cells was 8.3mM. At higher concentrations there was no differential response. Metabolism of L-HCA may account for the steeper dose response and greater potency of L-HCA compared to Glu, which can be shunted to many metabolic pathways. Additional evidence (publishing pending) suggests that Glu and L-HCA treatment increases susceptibility of the cells to peroxidative damage and that the mechanism for each of these compounds is identical. This research was funded by a grant from NINCDS #NS 14642.

286.9 IS THE DECREASED PC/PE RATIO IN RBC'S FROM SCHIZOPHRENICS DUE TO DECREASED SAM PRODUCTION? L.C. Tolbert, J.A. Monti and J.R. Smythies. Neurosciences Program and Dept. of Psychiatry, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

There is evidence which suggests that the erythrocyte membrane in schizophrenic patients has abnormal levels of certain phospholipids. In RBC's from schizophrenics phosphatidylserine (PS) levels appear to be elevated, while the levels of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) seem to be decreased as compared with normal controls. There are two mechanisms which might account for these results: 1) decreased decarboxylation of serine in the pathway PS → PE → PC, and/or 2) decreased production of a required substrate, i.e, SAM, for methylation of PE. We have also observed increased levels of PS and decreased levels of PC and PE in RBC's obtained from a small group of hospitalized schizophrenic patients. Further, we have confirmed the work of other investigators which suggests that methionine adenosyl transferase (MAT) activity in RBC's from schizophrenics is reduced, as compared with normal controls. Since alterations of cellular lipids can potentially affect a wide variety of physiological processes including receptor-stimulated cAMP formation, it is important to identify the mechanism responsible for the apparent abnormal ratios of membrane phospholipids in schizophrenics. We are currently investigating the possible correlation between decreased MAT activity and decreased ratios of PC/PE in erythrocytes obtained from schizophrenic patients. Results from these studies will be discussed in relationship with the current available data on phospholipid methylation.

286.10 Electron Microscopy of Cobaltous Chloride-induced Degeneration in the Rat Lateral Geniculate Nucleus. H.D.Schwark* and J.G.Malpeli. Neural and Behavioral Biology Program and Dept, of Psychology, University of Illinois, Urbana, ILL. 61801

Neurons are more susceptible to destruction by exposure to cobaltous chloride ($CoCl_2$) than myelinated fibers (Malpeli,accepted for publication). Thus, when small amounts of low concentrations of $CoCl_2$ are pressure-injected into the CNS, neuron cell death occurs while myelinated fibers are spared. To investigate the ultrastructural consequences of $CoCl_2$ injection, lesions were produced in the rat LGN by injecting 100 nanoliters of 4 mM $CoCl_2$ eight times, at one minute intervals, at one site. The rats were allowed to survive for 1½,2½,3½,7,10½,14,21,28, or 70 days, at which time the lesioned areas and the corresponding contralateral areas were prepared for electron microscopy. Light microscopic examination of these lesions revealed damaged neurons in an area of approximately 400μm radius around the site of injection.

The patterns of degeneration seen following $CoCl_2$ injection were very similar to those seen in the rat thalamus following decortication (Barron,et.al.,J.Neuropath.Exp.Neurol.,1973,32:218). Degenerating neurons were seen in the first 3½ days following the lesion. Dark degeneration appeared slightly earlier than pale degeneration, although both types were seen in the same tissue. Electron-dense postsynaptic profiles, probably dendrites of the degenerating neurons, were also evident at this time. The most common feature after 7 days was a population of large, swollen axons, both myelinated and unmyelinated, filled with mitochondria and assorted membranous profiles. Disrupted and empty myelin figures were characteristic of 10½ and 14 day survivals. By 28 days most degenerative processes appeared to have been completed. Large empty profiles, probably resulting from phagocytic removal of neuronal debris, were seen throughout the neuropil at this time. An unusual feature of the lesioned area at longer survival times (longer than 10½ days) was the appearance of aggregations of needle-like electron-dense particles. These aggregations may represent a novel degenerative process unique to $CoCl_2$-induced damage.

Large numbers of normal-appearing myelinated fibers were seen in the lesioned areas at all survival times, suggesting a significant sparing of axons-of-passage. In addition, normal-appearing synapses and dendrites (presumably originating outside the lesion) were seen at all survival times. It appears that injection of $CoCl_2$ may provide a method for localized destruction of neurons with minimal disruption of passing fibers. This would facilitate studies of, for example, the response of axon terminals to removal of their postsynaptic targets.

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286.11

WITHDRAWN

286.12 DISEASE EXPRESSION IN MOSAIC NERVES OF DYSTROPHIC ↔ SHIVERER MOUSE CHIMAERAS. A. Peterson, G. Bray and J. Marler*, Neurosciences Unit, Montreal General Hospital Research Institute and McGill University, Montreal, Québec, Canada.

Dystrophic mice (dy/dy and dy^{2J}/dy^{2J}) express two striking neural abnormalities: "patchy" basal laminas on myelinated Schwann cells throughout the PNS and,naked axons, typically restricted to longer spinal roots and cranial nerves. These morphological defects could be due to a primary effect of the Schwann cell's genotype or, could be a secondary consequence of mutant gene expression in some other cell type (Bunge & Bunge, J. Cell Biol. 78: 954-50, 1978). To investigate this issue, we have produced mouse chimaeras in which one cell line is genotypically dystrophic (dy^{2J}/dy^{2J}) while the other is non-dystrophic (+/+) but bearing the genetically unrelated shiverer mutation (shi/shi) in which myelin basic protein (P1) is deficient. Using PAP immunocytochemical techniques with 1 µm thick Epon sections, the presence of P1 can be detected in virtually all myelin in dystrophic PNS while myelin in shiverer PNS fails to react. Spinal roots and sciatic nerves from dy^{2J}/dy^{2J} ↔ shi/shi chimaeras contain two distinct populations of Schwann cells: those that react with P1 antibody (dy^{2J}/dy^{2J}) and the remainder that don't (shi/shi).

To determine if there is a correlation between the basal lamina phenotype and Schwann cell genotype, we have used serial sections (1µm thick for immunocytochemistry followed by ultrathin for EM analysis) permitting the genotype of those Schwann cells examined ultrastructurally to be identified. We have so far failed to detect a difference in basal lamina morphology between the dy^{2J}/dy^{2J} and shi/shi Schwann cells in chimaera sciatic nerves; rather, the percent of the Schwann cell plasma membrane covered in 171 µm of membrane from 26 dy^{2J}/dy^{2J} cells and 179 µm from 25 shi/shi cells reveals essentially intact basal lamina in both (96.9 ± S.E. 1.2 and 96.6 ± 1.2 respectively) compared with the control values of 82.9 ± 1.4 in $dy^{2J}/dy^{2J}$and 99.3 ± 0.5 in +/+ mice.

In the same chimaera relatively small bundles of naked axons are present but in affected L4 ventral roots both shi/shi and dy^{2J}/dy^{2J} Schwann cells are present and both are found in close proximity to naked axons.

Our results indicate that the basal lamina defect of genotypically dystrophic Schwann cells can either be corrected or fails to be expressed in the sciatic nerves of the chimaeras examined. The presence of naked axons in mosaic roots may indicate further that the genotype of the Schwann cell is not exclusively responsible for the failure of axon ensheathment in dystrophic spinal roots.

(Supported by M.D.A.C).

286.13 CRUSH INJURY CORRECTS DEFECTIVE BASAL LAMINA IN DYSTROPHIC MOUSE SPINAL ROOTS. G.M. Bray, S. David* , T. Carlstedt* and A.J. Aguayo. Neurosciences Unit, The Montreal General Hospital, Montreal, Que.

Dystrophic mice have prominent discontinuities of their Schwann cell basal lamina, both in the spinal roots where many axons are totally unensheathed and in more distal peripheral nerve segments where axon ensheathment and myelination are more complete (Madrid et al, 1975). Such basal lamina defects are reproduced in tissue culture and are corrected in vitro by the addition of normal fibroblasts (Bunge et al, 1982). We have examined the responses of the dystrophic Schwann cell basal lamina to experimental manipulations in vivo by unilaterally crushing one or more dorsal lumbo-sacral roots in C57 BL dy^{2J}/dy^{2J} and control C57 BL +/+ mice. After six weeks, the crushed and uncrushed roots were prepared for electron microscopy.

In roots that had been completely crushed, the characteristic groups of unensheathed axons did not redevelop. Although total numbers of axons were decreased, the regenerated nerve fibers were ensheathed as myelinated or unmyelinated nerve fibers. The morphology of Schwann cell basal lamina was examined and its distribution measured for groups of regenerated and uncrushed myelinated nerve fibers. Expressed as percentages of Schwann cell plasma membrane covered by basal lamina, the results were:

Group	Nerves	Fibers	Mean	Std. Error
+/+ uncrushed	3	43	99%	0.3
dy uncrushed	3	41	74%*	2.7
dy crushed	3	43	97%	0.6
+/+ crushed	2	28	99%	0.4

* significantly different, $p < 0.01$

Thus, the process of crush injury and regeneration appears to have corrected the basal lamina discontinuities in the dystrophic spinal roots. Because the regenerated dystrophic roots contain increased amounts of collagen, our results support the evidence from the tissue culture experiments that the extracellular matrix may be involved in the pathogenesis of the basal lamina disorder. However, the outcome of the present in vivo studies indicate that genotypically-normal fibroblasts are not required for this change to occur.

(Supported by the Muscular Dystrophy Association of Canada)

286.14 PATHOLOGY OF SKELETAL MUSCLE AND PERIPHERAL NERVE OF RATS TREATED WITH TRIETHYLTIN. E.A. Richman* and G.G. Bierkamper* (SPON: M. Rennels). Laboratory of Neuromuscular Toxicology. Division of Toxicology. The Johns Hopkins University, Baltimore, MD. 21205.

Trialkyltins are highly toxic compounds with widespread use in agriculture and industry. They are used, for example, as insecticides, fungicides, and as catalysts in the manufacture of plastics. Mammalian exposure to triethyltin (TET) produces hindlimb weakness, decreased acetylcholine release at the neuromuscular junction, decreased resting membrane potential of the soleus muscle, and edema and demyelination of the central nervous system. Involvement of structures of the peripheral nervous system has been controversial; hence, in the present study, light- and electron-microscopic observations were made to establish the extent to which peripheral myelin and axons are involved in the pathological response. Further, light microscopic examination of skeletal muscle was made to evaluate TET as a direct myotoxin. Although adult skeletal muscle is usually resistant to chemical toxins, decreased resting membrane potential and muscle weakness may result from direct damage to muscle fibers.

Adult, male Long Evans hooded rats (250-350g) were exposed to TET (30mg/L) in drinking water. After three weeks of exposure soleus and extensor digitorum longus (EDL) muscles were removed, weighed, frozen sectioned, and stained by routine histological procedures. Glutaraldehyde-fixed lumbar spinal cord, distal and ventral roots of spinal nerves L4, L5, and L6, and sciatic nerve were also removed and examined in paraffin sections and by electron microscopy.

Wet weight of soleus and EDL muscles of TET-treated animals was significantly reduced compared to normal and pair-watered controls; diameters of muscle fibers were reduced but no histopathology was seen. Spinal roots and distal segments of sciatic nerve showed abnormal myelin and axons. Myelin sheaths contained vacuoles which appeared to be formed by a splitting of the myelin at the intraperiod line while occasional axons, especially in the spinal roots, were enlarged or degenerated. These observations in the peripheral nervous system are consistent with findings of concurrent electrophysiological experiments in our laboratory showing decreased conduction velocity in sensory roots of sciatic nerve.

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286.15 ABERRANT NEURITE AND MEGANEURITE DEVELOPMENT IN A FELINE MODEL OF MUCOPOLYSACCHARIDOSIS (MPS)TYPE 1 AS REVEALED BY THE GOLGI METHOD. Steven U. Walkley and Mark E. Haskins*. Dept.Neuroscience, R.F. Kennedy Center, Albert Einstein Col. of Med., Bronx, NY 10461.

Recent studies using the Golgi staining technique in order to demonstrate neurons in their entirety have revealed that some types of neuronal storage disease, such as the gangliosidoses,represent far more than simple storage phenomena. Select types of neurons have been shown to undergo highly unusual morphological changes, including growth of aberrant neurites (dendrites) from axon hillocks and formation of spine-covered enlargements (meganeurites) interposed between somata and axonal initial segments. Formation of synapses on neurites and meganeurites also has been shown at the EM level, and such phenomena, taken as a whole, have been hypothesized to be the morphological substrate for neurobehavioral deterioration evident in these diseases.

The biomedical resource of animal models of neuronal storage disease offers a valuable opportunity to study such phenomena in detail. The present study concerns a 2½ year old male domestic short-haired cat which was derived from an MPS type 1 research colony at the Univ. of Pennsylvania. At the time of sacrifice multiple skeletal anomalies were observed but clinical neurological deterioration was not clearly evident. An absence of activity of the lysosomal hydrolase, α-L-iduronidase, had been demonstrated earlier in periperal tissues. The Golgi staining technique was applied to multiple areas of CNS and successful impregnations were achieved for cerebral cortex, hippocampus, caudate, thalamus, and cerebellum. A great variety of neurons were impregnated and all appeared to be of normal morphology except for select cells in cerebral cortex and hippocampus. In cortex, pyramidal neurons occasionally demonstrated axon hillock enlargement or the formation of meganeurites, and these often possessed spines or neurites. Other pyramidal neurons were normal or had small tufts of neurites projecting from otherwise normal axon hillock-initial segment areas. When meganeurites occurred they frequently exceeded adjacent somata in volume and meganeurite-associated neurites often had spines and thus resembled small dendrites. Regional cortical differences in the degree of meganeurite and neurite growth on pyramidal neurons were noted. In the hippocampus, pyramidal neurons most often displayed normal morphology but granule cells of the fascia dentata were seen which possessed aberrant basilar dendrites and/or numerous spines on axon hillock-initial segment areas.

The above described morphological changes have now been documented in 5 types of neuronal storage disease (GM1 and GM2 gangliosidosis, α-mannosidosis, sphingomyelin lipidosis, and MPS type 1), but an explanation for their development and significance remains unknown.(Supported by NS-07512,AM-25259 & GM-20138)

286.16 β-MANNOSIDOSIS: LESIONS OF THE DISTAL PERIPHERAL NERVOUS SYSTEM. J. A. Malachowski* and M. Z. Jones. Department of Pathology, Michigan State University, E. Lansing, MI 48824.

β-Mannosidosis, a recently described autosomal recessive glycoprotein metabolic disease, is characterized by deficiency of β-mannosidase, accumulation and excretion of oligosaccharides, numerous neurovisceral cytoplasmic lysosomal storage vacuoles, central nervous system axonal spheroids, and dysmyelinogenesis. Lesions in the peripheral nervous system appeared to be confined to Schwann cell cytoplasmic vacuolation and isolated axonal dense bodies. Examination of the gingiva revealed other peripheral nerve lesions. Alterations in the sensory endings included vacuolated Merkel cells and their associated enlarged, spherical neural end plates containing dense bodies. Free-ending axons in the prickle cell layer of the epithelium were enlarged and contained dense bodies. Pacinian corpuscles in the lamina propria were often enlarged and associated with dense body accumulation in related axons. Several types of abnormal axons were present in the lamina propria. These included giant demyelinated axons with accumulations of dense bodies, enlarged myelinated axons with abundant dense bodies and unmyelinated axons with dense bodies. Axonal enlargement and loss of myelin were noted only with dense body accumulation. The pathogenesis of the axonal and myelin lesions in the distal peripheral nerves and the central nervous system in β-mannosidosis remains to be clarified, but these preliminary studies suggest a relationship between axonal enlargement, dense body accumulation, and myelin loss. Supported by NS-16886 to MZJ.

286.17 COMPARISON OF THE RELATIVE RATES OF PROTEIN SYNTHESIS AND DEGRADATION IN NORMAL AND DYSTROPHIC CHICKEN MUSCLE, B. A. Wolitzky*, H. L. Segal* and M. S. Hudecki (SPON: R. R. Almon). Division of Cell and Molecular Biology, State University of New York, Buffalo, NY 14260.

Two experimental approaches have been used in studying protein turnover in the dystrophic chicken (Line 413, U. Calif., Davis) and in its genetically-related control (Line 412). Using primary muscle cultures established from 12-day embryonic breast muscle, the rates of synthesis/degradation of homogenate, soluble and myofibrillar proteins have been measured. Specific contractile proteins studied include among others myosin, α-actinin, actin, tropomyosin, etc... Degradation rate measurements were carried out under conditions where isotope reincorporation was excluded. The overall rates of protein synthesis as well as the rate of incorporation of ^{35}S-methionine into specific myofibrillar proteins were not significantly different between the normal and dystrophic genotypes. Myofibrillar proteins of both culture types were synthesized at a rate slower than average cell proteins. Degradation rates were exponential for at least one half-life yielding half-life values of 34 h in both cases. Degradation of specific myofibrillar proteins were determined by the decay of radioactivity from specific proteins isolated by SDS-PAGE from cultures during various degradation times. The muscle protein components exhibited heterogeneous rates of degradation, and were found to be similar in both culture types. The influence of leupeptin and 'step-down' conditions on protein degradation have appeared elsewhere (Wolitzky et al., Exp. Cell Res. 137:295, 1982).

A second approach involved the measurement in vitro of the rates of incorporation of ^{3}H-tyrosine into isolated, intact anterior (ALD) and posterior (PLD) latissimus dorsi muscles from normal and dystrophic chickens of various ages. In 30- and 60-day old birds, the rates of protein synthesis in the PLD (fast-twitch) were 1.5 x and 2.5 x faster in the dystrophic muscles, resp. Rates of protein synthesis were similar in the ALD (slow-twitch) muscles of both genotypes. The dystrophic PLD also exhibited a progressive decline in mg noncollagen protein/g wet weight, reaching 18% decrease by 60 days ex ovo. These findings support the concept of a fiber-type specificity in avian dystrophy, with the slow-twitch ALD muscle apparently spared. This study is supported by grants from the Muscular Dystrophy Association and NIH. The technical help of C. M. Pollina is gratefully acknowledged.

286.18 PEROXIDATIVE DAMAGE MAY MEDIATE L-GLUTAMATE INDUCED CELLULAR DEGENERATION OF CULTURED FIBROBLASTS. P.C. May and P.N. Gray. Dept. of Biochemistry & Molecular Biology, Univ. of Oklahoma HSC, Oklahoma City, OK 73190.

A current hypothesis suggests L-glutamic acid (Glu) may be the degenerative agent responsible for the neuropathology seen in Huntington's Disease (HD). Neuronal degeneration associated with HD results in severe atrophy of the corpus striatum, an area receiving glutamatergic input via the corticostriatal tracts. HD skin fibroblasts in culture are more sensitive to the effects of Glu and undergo cellular degeneration following exposure to 15-20mM Glu for 24 hours (Gray,P.N. et al, 1980, BBRC 95:707). Control fibroblasts undergo similar degeneration but only at higher Glu concentrations (>35mM). L-homocysteic acid (HCA), a sulfonic acid analog of Glu, at 30mM induced cellular degeneration in HD and control cells morphologically indistinguishable from Glu induced death. This suggests a common toxic mechanism may be operative in both HD and control cells but initiated at a lower Glu concentration in HD cells. Experimentation to elucidate the toxic mechanism has provided results indicating peroxidative damage ultimately may be responsible for Glu induced cell death. Cystine (Cys), an essential amino acid for cells in culture, is routinely present in culture media (DMEM) at 0.2mM. At that Cys concentration, 30mM Glu inhibited Cys uptake by 55-60% in both HD and control cells. Cells cultured in media with 0.1mM Cys (MEM) exhibited greater sensitivity to Glu and L-HCA. Supplementing MEM with Cys up to 0.3mM reduced Glu and L-HCA induced death from 80% to only 5%. Cystine, as cysteine, normally is incorporated rapidly into glutahione (GSH). Eight hour treatment of HD and control cells with 30mM Glu or 10mM L-HCA resulted in an 80% decrease in total GSH levels. Similar treatment with 30mM Asp or 10mM D-HCA, both noncytotoxic, had no effect on intracellular [GSH]. Supplementation of MEM with Cys significantly increased intracellular GSH in cells treated with Glu or L-HCA. Glutathione has a protective function in cells by maintaining a proper intracellular redox environment. Decreases in cellular GSH could result in an increasingly oxidized environment promoting peroxidative activity. Accordingly, Glu induced cell death was blocked by addition of various antioxidants (BHA, Vitamin E, ethanol). These results indicate Glu induced cellular degeneration may be due to a toxic mechanism initiated by glutamate's inhibition of Cys uptake. Since no major differences were noted in HD and control Cys uptake or GSH levels following Glu treatment, the differential sensitivity of HD fibroblasts to Glu may be due to an altered susceptibility to peroxidative damage. This research was funded by a grant from NINCDS #NS 14642.

286.19 A THIAMIN PYROPHOSPHATE DEPENDENT PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY IN A NEWBORN WITH CONGENITAL LACTIC ACIDOSIS, Lois M. Hinman* and John P. Blass, Burke Rehabilitation Center, Cornell Medical College, White Plains, New York 10605

Congenital lactic acidosis is a symptom of a number of metabolic abnormalities including pyruvate dehydrogenase complex (PDHC) deficiencies. There have been reports of congenital defects in all three catalytic subunits of PDHC and in PDHC activation by phosphatase (Evans, O. Arch Neurol 38:515-519, 1981). A few thiamin dependent PDHC deficiencies have also been studied in cultured skin fibroblasts using [1-^{14}C]pyruvate decarboxylation assays (Wick, et al Agents and Actions 7:405-410, 1977; Stumpf, et al Neurol 4, 1982).

We now report reduced PDHC activity, and an abnormally large requirement for thiamin pyrophosphate (TPP) in an infant whose neonatal death at five days followed severe metabolic acidosis, in a course similar to that of a sibling. In these studies, PDHC was fully activated in cultured skin fibroblasts using dichloroacetate (DCA) activation and measured with an arylamine acetyltransferase-coupled spectrophotometric assay. In normal control fibroblasts, DCA-activated PDHC, assayed in the presence of 0.2 mM TPP, gave an activity of 4.61 ± 0.38 nmoles/min/mg protein (n = 4) and without TPP the average activity was 4.21 ± 0.60. PDHC activity in DCA-activated fibroblasts from the patient was 1.62 ± 0.25 nmoles/min/mg protein but only 0.61 ± 0.27 in the absence of exogenous TPP, a value even lower than we normally found for unactivated PDHC in normal controls and approximately that found for his cells without DCA activation. A preparation of PDHC partially purified from the patient's heart tissue showed no activity without adding TPP to the assay mixture, in contrast to normal controls which showed little TPP requirement. TPP binding to mammalian PDHC has been shown to promote PDHC activation (Roche and Reed, BBRC, 48:840, 1972). Our results emphasize the importance of TPP binding for proper function of PDHC, a protein now thought to be involved in synaptic plasticity, in human tissue.

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286.20 MAST CELL DEGRANULATION IN RATS DURING ACUTE SOMAN TOXICATION, J. Doebler*, A. Anthony*, T. Bocan* and T.-M. Shih (SPON: J. H. McDonough, Jr.). Penn State Univ., University Park, PA 16802 and U.S. Army Med. Res. Inst. of Chem. Def., APG, MD 21010.

Mast cell responses of Sprague-Dawley X Wistar rats were analyzed at 3-10, 30, 60 and 120 min intervals following sublethal and lethal injections of soman (65, 120, or 195 μg/kg, s.c.). Scanning-integrating cytophotometry of azure B and toluidine blue O stained mesentery preparations was used to quantify extent of degranulation of individual mast cells; plasma and erythrocyte cholinesterase levels were monitored to assess the extent of acetylcholinesterase (AchE) inhibition. A marked, dose-dependent depletion of mast cell granules was evidenced as early as 3-10 min following soman poisoning. Similar cellular response patterns were also evidenced in stromal compartments of the lung, liver and salivary glands, indicating that AchE inhibition is associated with a massive and generalized degranulation of mast cells throughout the body. These observations indicate that an anaphylactoid-type response appears to be an important aspect of soman toxication which probably potentiates acetylcholine-mediated bronchiolar constriction and respiatory depression. (Supported in part by USAMRDC Grant DAMD 17-81-C-1202).

286.PO DOSE AND TIME-COURSE EFFECTS IN NEUROPATHOLOGY PRODUCTION IN RATS FOLLOWING TRIMETHYLTIN INTOXICATION. L.W. Chang, D. Woolley*, and L. Zimmer*. Dept. of Pathology, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205; Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

Young adult, female Sprague-Dawley rats were used in the experiment. Animals were subjected to a single oral administration of trimethyltin (TMT) chloride at dosage levels of 7.5 or 25 mg/kg b.w. Control animals were administered with equal volumes of saline solution. Animals treated with 25 mg TMT/kg b.w. showed tremor as early as 48 hours following TMT exposure and were sacrificed via intracardial perfusion with 2.5% glutaraldehyde at days 2, 3 and 6. Rats treated with 7.5 mg TMT/kg b.w. also showed slight head tremors by the 5th day. However, these animals appeared normal again by 21 days. These groups of animals were sacrificed at days 5 and 21. Brains from all the animals were removed carefully and further fixed in 10% buffered formalin, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin-eosin for histopathology investigation. Light microscopic examination revealed that 2-3 days after treatment with 25 mg TMT/kg b.w. (acute dose), there was extensive neuronal necrosis in the dentate granule cells and in the pyriform and entorhinal cortices. Significant cellular necrosis was also observed in the hippocampal proximal CA_3 pyramidal neurons and in the polymorphic area of the dentate hilus. By the 6th day of intoxication, while there was a decreased number of necrotic cells in the dentate granular layer, increased pyramidal cell necrosis occurred in the $CA_{1,2}$ areas of the hippocampal formation. Prominent cellular necrosis was still observed in the proximal CA_3/dentate hilus areas and in the pyriform/entorhinal cortices. In contrast, at the 5th day of a lower dose (7.5 mg/kg) exposure, animals demonstrated only minimal neuronal damage in the pyriform/entorhinal cortices and in the proximal CA_3/dentate hilus areas. After 21 days of intoxication, extensive neuronal necrosis were found in the pyriform/entorhinal cortices and in the proximal CA_3 area. Increased gliosis and microglial infiltration in the dentate hilus area was also observed. At this time of intoxication, neuronal necrosis was also detected in the dentate granule cells and in the $CA_{1,2}$ pyramidal neurons of the hippocampal formation. Cellular damage to the amygdaloid nucleus was only minimal and inconsistent. Our present investigation demonstrated that under acute toxic conditions, dentate granule cells and pyriform/entorhinal cortical neurons were most vulnerable to the toxicity of TMT. Increased pyramidal cell damage occurred in a later time. Continued cellular damage was also observed in rats, showing increasing and persistent neuronal necrosis in various areas of the limbic system even 21 days after the toxic exposure, denoting the long-term toxic effects of TMT to the nervous system.

287.1 PHOSPHORYLATION OF BRAIN PROTEINS FOLLOWING ACUTE AND CHRONIC ELECTROCONVULSIVE AND PENTYLENETETRAZOLE CONVULSIONS.
M.Horan*, J.G. Bajorek*, and A.V. Delgado-Escueta. Dept. Neurology, School of Medicine, Univ.Calif.Los Angeles. L.A., CA.90024.

The phosphorylation of synaptic brain proteins has been postulated to regulate many neuronal functions including transmitter release and postsynaptic responses. In epilepsy, alterations in these mechanisms may be responsible for changes in excitability. We evaluated the state of phosphorylation of a protein or group of proteins with approximate M.W. of 18,000 daltons, postulated by Erhlich (J. Neurochem. 34:1327-1330, 1980) to be involved in seizures.

Rats convulsed acutely (1 tonic-clonic seizure) or chronically (1 tonic-clonic seizure on 6 consecutive days) with electric shock (ES) (ear clip electrodes, 150ma, 0.2 sec duration) or with pentylenetetrazole (PTZ), (70 mg/kg i.p.) were decapitated during convulsions or 24 hours later. The submitochondrial fraction (P_2) of cerebral homogenates was incubated in vitro with (P^{32}) ATP and various concentrations of Mg^{2+} and Ca^{2+} ions. Aliquots were electrophoresed on SDS-polyacrylamide slab gels. Gel proteins were stained with Coomassie Blue and then autoradiographed. Densitometric scans were integrated to yield quantitative data on phosphate incorporation.

The slab gels revealed the phosphorylation of 14 proteins, one of which (MW.18K) was most noticeably altered by convulsions. Both ES and PTZ convulsions (1 or 6 days) increased in vitro phosphate incorporation in the 18K protein (ES-12% to 65%, PTZ-30% to 150%, dependent on incubation conditions). However, 24 hours following a single convulsion, P^{32} incorporation returned to control levels. One day following chronic convulsions, incorporation was reduced but remained (7.2%-109%) above control values. PTZ convulsions were twice as effective as ES convulsions at stimulating phosphorylation. Ionic conditions also affected incorporation. Increasing Mg^{2+} concentrations (0-10 mM) increased P^{32} incorporation, while increasing Ca^{2+} (0-10 mM) decreased P^{32} incorporation. It was under conditions of high Ca^{2+} (10 mM) and high Mg^{2+} (10 mM), that the greatest difference (150%) from control in enhanced P^{32} incorporation was attained. We interpret these findings to mean that under different convulsive parameters a common protein alters its phosphorylative state and that the alteration persists after repeated convulsions. This protein (18K) or group of proteins may be involved in mechanisms which later lead to epileptic sequelae.

287.2 FOCAL NEOCORTICAL SEIZURES CAUSE DISTANT LESIONS WITHIN SEIZURE PATHWAYS. J. Labruyere*, J.W. Olney and R.C. Collins (SPON: S. Eliasson). Depts. Psychiatry, Pathology and Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We applied several convulsants (bicuculline, penicillin, picrotoxin, folic acid or physostigmine) topically to the rat sensorimotor cortex in concentrations sufficient to cause repetitive focal convulsions of the contralateral forelimb. Animals were alert and breathed normally throughout. After 3 hr or more of seizure activity, we transcardially perfused the brains with buffered aldehydes for light and electron microscopic analysis. We found similar neuropathological changes regardless of the convulsant used. In sectors of ipsilateral ventrolateral and ventrobasal thalamus connected somatotopically with the cortical focus, we found marked swelling of dendrites and either swelling or dark cell degeneration of neuronal cell bodies. Since this is the type of cytopathology induced by local injections of the excitotoxic amino acid glutamate (Glu), and Glu is thought to be the transmitter of cortical pyramidal neurons, we suggest these changes occur as a result of excessive epileptic-induced release of Glu from corticothalamic pyramidal axons. In the cortex there was a distinct band of dark cells and spongiform changes confined to layer IV lateral and posterior to the focus in sensory cortex. The dark cell changes, confined primarily to interneurons, may be due in part to release of Glu from pyramidal collaterals. The spongiform changes consisted of marked dilatation of axon terminals synapsing with normal dendritic spines. Because of the laminar profile of these changes, the axospinous synaptic relationship, and the involvement of thalamocortical relay neurons in thalamus we conclude these swollen intracortical terminals are those of thalamic neurons.

In summary, we find that repetitive focal cortical seizures cause acute degeneration of specific neuronal elements within seizure pathways. There is no basis for attributing the damage to hypoxemia in this model; rather we propose that continuous discharge activity and excessive release of the excitotoxic transmitter, Glu, provides a likely explanation for the cytopathology observed in thalamus and cortex. The swelling of intracortical thalamic axon terminals may relate to the fact that these specific axonal elements are intensely driven ortho- and antidromically during focal neocortical seizures. Supported by grants from the Epilepsy Foundation of America and USPHS (NS-09156, RSA MH-38894 to JWO; NS 14834 to RCC).

287.3 EPILEPTOGENIC EFFECTS OF SMALL HIPPOCAMPAL LESIONS: RELEVANCE OF IRON-DEPOSITION. Kenneth A. Campbell, Barry Bank*, and N. W. Milgram. Dept. of Psychology, University of Toronto, Scarborough College, West Hill, Ontario, Canada.

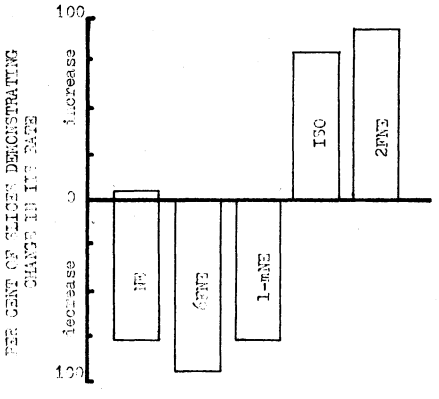
Ten rats with stainless steel or platinum/irridium electrodes received small (.5-1 mm dia) unilateral lesions to the dorsal CA3 region of the hippocampus by passing direct current with the hippocampal electrode as the anode (+) and a rectal cathode. Parameters were either 2.0 mA for 20 sec or .5 mA for 80 sec. EEG recording was monitored from a contralateral CA3 electrode continuously for up to 18 hrs prior to the lesion and up to 48 hrs after the lesion, with subsequent 24-hr records taken after 3-12 days.

Lesions through steel electrodes were produced in 6 animals; 5 of these demonstrated recurrent epileptiform discharges, analogous to the primary and secondary afterdischarges (ADs) which can be produced in the hippocampus by electrical stimulation. Such discharges occurred as early as 14 min after the lesion and recurred up to 4/hr for as long as 6 hrs. In the two most extreme cases, the AD-like discharges developed into continuous spiking (.5/sec), lasting over 6 hrs. High frequency epileptiform activity subsided in most cases within 24 hrs, although spontaneous isolated spikes continued to occur. Epileptic activity was associated with a profound behavioral freezing reaction, and one clonic seizure was observed 10 days after the lesion. Prussian Blue staining confirmed the deposit of iron ions in these animals, with the most severe case being associated with the additional presence of blood clots. Lesions produced through platinum electrodes (2 mA) did not result in any clear epileptic effects.

The efficacy of lesions using only .5 mA through a steel electrode, and the absence of effect with platinum electrodes suggest that deposit of iron ions may be a critical factor. This observation is consistent with reports that microinjection of iron salt solutions into cortex causes seizures (Willmore et al., Brain Research, 152, 406, 1978). Electrolytic lesions with steel electrodes have also been reported to produce seizure activity following extensive damage to the entorhinal cortex (Dasheiff & McNamara, Brain Research, 231, 444, 1982). These findings have important implications for the interpretation of studies employing lesions of the hippocampal region.

287.4 NORADRENERGIC MODULATION OF HIPPOCAMPAL EPILEPTIFORM ACTIVITY IN VITRO. Alan L. Mueller and Thomas V. Dunwiddie. Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver CO 80262.

The role of l-norepinephrine (NE) in regulating epileptiform burst discharges was examined in the in vitro rat hippocampal slice preparation. Interictal spikes (IIS) were generated by superfusion of slices with medium containing sodium penicillin-G (2000 U/ml) and elevated levels of potassium (8.25 mM), and were recorded extracellularly in region CA1. Exogenously applied NE had anticonvulsant properties in that it suppressed IIS rate in nearly every slice studied. This anticonvulsant property of NE was shared by the alpha receptor agonists 6-fluoro-NE (6FNE), l-alpha-methyl-NE (l-mNE) and clonidine (clon), but not by d-alpha-methyl-NE (d-mNE) or l-phenylephrine. The beta agonists 2-fluoro-NE (2FNE) and l-isoproterenol (ISO), on the other hand, were proconvulsant in that they increased IIS rate. Anticonvulsant responses were selectively antagonized by the alpha antagonist phentolamine, while proconvulsant activity was selectively antagonized by the beta blocker timolol. Taken together, these results suggest that alpha and beta receptor activation may inhibit and enhance, respectively, epileptiform discharges in the rat hippocampus.



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287.5 ADRENERGIC RECEPTORS IN AUDIOGENIC SEIZURE SUSCEPTIBLE MICE. C. Nyquist-Battie, C. Lints, R. Fortney* and A. Gochee*. Depts. of Biol. Sci. and Psychology, Northern Illinois Univ., De Kalb, IL 60115.

Alteration in noradrenergic transmission has been suggested to contribute to audiogenic seizure (AGS) susceptibility in DBA/2J mice based on studies of adrenergic ligand effects, norepinephrine levels and turnover in AGS mice, although the results of these studies have been contradictory. To determine if Beta-adrenergic receptors are altered in AGS, the following study was undertaken. After AGS and control (C57BL/6) mice were decapitated at 20-22 days of age, cerebrum, midbrain and cerebellum were excised. Regions from 2-6 animals were pooled. A washed membrane fraction (15,000 x g- 30 min) was prepared and was resuspended in 50 mM Tris-HCl pH 8 with 0.1% ascorbic acid and 1 μM pargyline. Incubations in triplicate were carried out for 30 min with 0.5-25 nM (3H)- Dihydroalprenolol (DHA). Non-specific binding was that in the presence of 0.1 mM (-) alprenolol. Bound ligand was separated from free by filtration through Whatman GF/B filters with 2 X 7 ml cold buffer washes. Counting was performed in 10 ml Formula 947 (NEN). Scathard analysis of data was performed using a least squares linear regression computer program.

Results of the above show that altered densities of beta receptors exist in the midbrain but not in other areas of AGS mice, although no differences in affinities in any area were seen. In cerebral samples no difference between strains were observed in apparent K_D (1.23 ± .18 nM) or in densities (5.35 ± .09 pmole DHA/ gm wet weight). Similarly, no strain differences were seen in either parameter in the cerebellum (0.099 ± .008 nM; 0.525 pmoles DHA/ gm weight). Although no change in apparent K_D (0.716 ± .014 nM) was seen in the midbrain of the AGS mice, a 35% increase in receptor density was observed (.272 ± .031(C57) vs. .367 ± .029(DBA) pmole DHA/ gm weight). Studies now underway to determine if the increased densities are due to either beta-1 or beta-2 receptors or both.

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287.6 ELECTROENCEPHALOGRAPHIC AND EPILEPTOGENIC EFFECTS OF CORTICOTROPIN RELEASING FACTOR (CRF) IN RATS. C.L. Ehlers*, S.J. Henriksen, F.E. Bloom, J. Rivier**, and W.J. Vale**. A.V. Davis Ctr. and Peptide Biology Laboratory**, The Salk Institute, La Jolla, CA 92037.

Evidence from clinical and experimental studies have suggested that a disruption of the pituitary-adrenal hormonal axis may lead to electroencephalographic (EEG) abnormalities as well as enhanced sensitivity to convulsive seizures. In the present study we investigated the actions of the recently sequenced neuropeptide corticotropin releasing factor (CRF) on the EEG of rats. Male Sprague-Dawley rats were stereotaxically implanted with bipolar electrodes in the dorsal hippocampus (DHPC), amygdala (AMYG), cerebral cortex (CTX) and with a cannula in the lateral cerebral ventricle. Two weeks later rats were individually adapted to the recording chamber and injected (intracerebroventricularly, i.c.v.) with 15 μl of saline following which EEG and behavior were recorded for 2 hours. Immediately following these control recordings rats were injected i.c.v. with CRF (0.01-100 ug) and continually monitored for periods up to 8 hours. In addition to polygraphic recordings the EEG was stored on tape for power spectral analysis.

ICV administration of saline was associated with behavioral quiescense and EEG signs of sleep. Administration of low doses (10 ng) of CRF produced after a delay of 15-20 minutes EEG activation associated with increased 6-8 HZ activity in the DHPC and a decreased low frequency (<15 HZ) activity in CTX. Time series analysis of individual spectral bands revealed an increase in stability of DHPC and CTX EEG patterns following CRF. These effects of low CRF doses were not associated with notable increases in locomotor behavior. ICV doses of .1-1 ug produced a long lasting EEG activation associated with increased locomotor and grooming behavior. In this dose range, after a delay of approximately 2 hrs some rats developed large interictal spikes occurring first in the AMYG but later spreading to DHPC. Handling of these rats or sudden loud sounds could frequently produce AMYG afterdischarges associated with behavioral arrest and wet dog shakes. At doses of 10-100 ug CRF produced intense behavioral activation consistently associated with AMYG interictal spikes and afterdischarges. In addition after a delay of 4-7 hrs, motor seizures developed in a number of rats. These seizures spontaneously progressed in a manner indistinguishable from those produced by AMYG "kindling" stimulations. These studies suggest a spectrum of CNS functions for CRF from regulation of brain excitability to modulation of temporal lobe seizures. (Supported by the Klingenstein Foundation and AM 26741.)

287.7 SPECIFIC BINDING OF [^{3}H]-L-GLUTAMIC ACID IN THE HIPPOCAMPUS OF KINDLED RATS. J.T. Slevin and L. Ferrara*. VA Med. Ctr., Depts. of Neurology and Pharm. and Sanders-Brown Aging Ctr., Univ. of Ky., Lexington, KY. 40536

Kindling, the induction of persistant generalized seizures by subthreshold electrical stimulation, is accepted as a model of human epilepsy. Though kindling is a transsynaptic process (Messenheimer and Steward, Exp. Neurol., 1979), changes in various neurotransmitter agents have generally been considered compensatory and not causal. This study investigated changes in glutamic acid receptor binding during the process of electrical kindling.

Male Sprague-Dawley rats (200-225 gms.) were permanently implanted with bipolar electrodes into the right entorhinal cortex. The electrodes were switched from a continuously recording polygraph (head screw in frontal sinus as reference electrode) to a constant current stimulator with an electrical switching box. After surgery, the after-discharge (AD) threshold was determined for each rat by stimulating through the electrodes (1 sec. train of 60 Hz biphasic symmetrical square waves of 1 msec. pulse duration) with increasing current intensities until an AD was recorded or 1000 μA attained. Rats were kindled once daily for 1 sec. at the experimentally-determined AD threshold (average, 550 μA). Kindling was continued until Stage V seizures (Racine, EEG and Clin. Neurophys., 32: 281, 1972) were elicited on two consecutive days (second seizure by 22.5±0.5 days).

Animals were sacrificed within 24 hours after the last convulsion; the brain was quickly removed and rapidly dissected at 2°C. Specific binding of [^{3}H]-L-glutamic acid ([^{3}H]-glu) to membranes isolated from left hippocampus and cerebellum of rats either kindled by entorhinal stimulation, given electroshock seizures through ear clips or receiving no electrical current was measured. External Ca^{++} added to the membrane preparation increased [^{3}H]-glu binding in hippocampus as previously described (Baudry & Lynch, Science, 1981) but not in cerebellum. Binding of [^{3}H]-glu to hippocampal membranes contralateral to the side of entorhinal kindling stimulation (9.3±0.5 pmoles/mg membrane protein) was significantly elevated ($P<0.01$) compared to nontreated controls (6.9±0.6 pmoles/mg membrane protein). The most parsimonious explanation for these observations would implicate functional alteration in putatively glutamatergic perforant and commissural pathways. It is noteworthy that CA3 pyramidal cells, whose axons comprise the hippocampal commissural path, are the source of interictal spikes or paroxysms of epileptic discharge in three quite dissimilar models of epilepsy in addition to possibly this kindling model. Supported by Research Grant from the Veterans Administration.

287.8 CLINICAL AND METABOLIC ALTERATIONS INDUCED BY SYSTEMIC INJECTION OF KAINIC ACID IN IMMATURE RATS. E.Tremblay* and Y.Ben-Ari. Lab. Physiologie Nerveuse,CNRS, 91190 Gif-sur-Yvette, France.

In adult rats,systemic injection of Kainic acid (KA) produces a syndrome in which electrographical and metabolic datae suggest that limbic structures occupy a central position (Ben-Ari et al, Neurosci., 6:1391,1981; Lothman and Collins,Brain Res.,218:299, 1981).Furthermore,although the ammon'horn,entorhinal and subicular cortices are activated first electrographically and metabolically (as visualized with the 2 deoxyglucose (2DG) method), it is only at a later stage (1_2hrs),when the amygdaloid complex and closely related structure (medial thalamic nucleus and limbic cortex) are activated,that the animals display motor signs which are seen following "kindling" and are typical of limbic epilepsy in adult rats (see Racine,Electroenceph.Clin.Neurophysiol.,32:281, 1972).

Since a) following systemic (or intra-amygdaloid) KA the rats display spontaneous limbic seizures after long delays (unpublished results) and b) the pathological alterations, like those seen in post mortem studies of chronic epileptics,are largely restrited to these limbic structures,it has been suggested that this procedure constitutes a useful model to study the relationship between epilepsy and brain damage (Ben-Ari et al,ibid).However,it is also well known that hippocampal sclerosis is often also associated with convulsions occuring during childhood (for instance in relation to fever) and which can give rise to epilepsy.We have therefore investigated the sequelae of systemic KA in pup rats.

In immature rats (3-13 days) systemic KA produces readily (10-35 min.) typical tonico-clonic generalized convulsions somewhat reminiscent of those seen after bicuculline or pentetrazole injections in adult rats (Ben-Ari et al., ibid) or hyperthermia in pups (Holtzman et al., Science, 213 : 1034, 1981). None of the limbic signs are elicited. The 2DG studies reveal an increase in metabolism restricted to part of the hippocampal formation (i.e. CA3 and CA1 but not the subicular or enthorhinal cortices) as well as the lateral septum, but not other limbic structures (notably the amygdala). The limbic signs approximately occur at 14-15 days (wet-shakes) and typical full limbic motor seizure at 21-23 days.

These observations a) add further support to the notion that activation of the amygdaloid complex is a prerequisite for limbic motor seizures, b) indicate that the CA3 layer of the hippocampus is particularly susceptible to KA in pups as it is in adult rats and c) raise the problem of the anatomical correlates of the convulsions seen in immature rats. Further experiments are currently performed to see whether these convulsions, as febrile convulsions in infants, can give rise, at longer delays, to chronic epilepsy.

287.9 MORPHINE ENHANCEMENT OF KAINATE NEUROTOXICITY IS OPIATE RECEPTOR SPECIFIC. T.A. Fuller, S. Buchsbaum*, and J.W. Olney. Dept. Psychiatry, Washington University Sch. Med., St. Louis MO 63110.

Subcutaneous administration of kainic acid (KA) produces a neurotoxic syndrome consisting of limbic convulsions thought to originate within the hippocampus and brain damage largely localized within the limbic system. While pretreatment with the anticonvulsant diazepam markedly suppresses both the convulsions and brain damage induced by KA, morphine augments both phenomena. It is postulated that KA-induced convulsions underlie a substantial portion of the KA-induced neuropathology and that morphine, which disinhibits the hippocampus, enhances the KA neurotoxic syndrome by decreasing the seizure threshold in limbic circuits. Since morphine exerts an anticonvulsant effect against the generalized seizures induced by pentylenetetrazol or fluorothyl, the following experiments were performed to determine if morphine's proconvulsive effect upon KA toxicity is an opiate receptor specific action.

Male Sprague-Dawley rats weighing 200-300 g were injected sc with H_2O or one of several opiate agonists 10 min prior to KA (6.5 mg/kg); in other experiments, rats were injected with naloxone 5 min prior to morphine (10 mg/kg) and 15 min prior to KA (6.5 mg/kg). The rats were observed for convulsions for at least 2 hr and then sacrificed by aldehyde transcardial perfusion at 4 or 24 hr after injection to permit histological evaluation of the brains. Morphine, levorphanol, etorphine and ketocyclazocine significantly enhanced the epileptogenic potential of KA in a dose-related manner; whereas H_2O/KA produced convulsions in 10.6% of the rats, the opiate/KA combinations produced convulsions in 50% of the rats at doses of 1.39×10^{-5}, 4.5×10^{-6}, 5.3×10^{-9} and 7.2×10^{-6} moles opiate/kg respectively. The opiate/KA treated animals which convulsed revealed the typical pattern of KA-induced pathology in the hippocampus, thalamus,amygdaloid complex and olfactory cortex. Those which did not convulse revealed no brain damage. Neither dextrorphan (10-20 mg/kg) nor SKF-10,047 (10-40 mg/kg) enhanced KA neurotoxicity. Naloxone (20-0.5 mg/kg) blocked the enhancement of KA neurotoxicity by morphine in a dose-dependent manner with the blocking effect being lost when the dose was lowered to 0.01 mg/kg.

The enhancement of KA toxicity by morphine meets major criteria for an opiate receptor specific action--the effect is reproduced by other opiate agonists according to their relative opiate potencies, it is not reproduced by a dextrorotatory opiate, and it is blocked by naloxone. Regarding the subtype of receptor involved, our data primarily implicate the mu receptor, but the possible involvement of the kappa or delta receptor requires further study. Supported by USPHS grants DA-00259, RSDA MH-00330 (TAF) and RSA MH-38894 (JWO).

287.10 TIME-COURSE OF EFFECT OF GAMMA-VINYL GABA (GVG) ON CONVULSANT MODELS IN RODENTS. Gary D. Novack, Francis P. Miller and Judith I. Allen* Merrell Dow Research Center, Cincinnati, OH 45215

GVG, an enzyme-activated irreversible inhibitor of GABA transaminase, elevates whole brain GABA levels in mice (Jung et al., J. Neurochem. 29:797, 1977). We recently reported that GVG pretreatment prolonged the latency to picrotoxin-induced seizures in mice, and that the time-course of this effect paralleled the whole-brain GABA levels (Soc. Neurosci. Abs. 7:110, 1981).

In the present study, we investigated the time-course of effect of GVG in three additional chemical and two electrical models of epilepsy in mice, the effect of GVG in the multi-electroshocking (ECS) paradigm of Gale and Iadarola (Science 208:288, 1980), and in a dual-ECS paradigm.

GVG delayed the onset to convulsions in bicucilline, 3-mercaptopropionic acid and pentylenetetrazol-induced seizures in mice, with a maximal effect 4 to 18 hr after treatment (ED50's 200-1000 mg/kg i.p.). GVG increased the duration of tonic hindlimb extension in maximal electroshock seizures in mice, with a maximal effect 4 hr after treatment (400-1600 mg/kg). No significant effect was seen at any time point up to 72 hr on the electrical threshold for extension in mice at doses up to 1600 mg/kg.

In the multi-ECS paradigm, GVG elicited an increase in tonic hindlimb extension time in both rats and mice at 12 hr after treatment. In mice, extension durations remained elevated for the next 24 to 48 hr. However, in rats, a significant dose-related decrease in extension duration was seen at 60 hr after treatment. No effect of GVG on extension duration was seen in rats shocked 108 hr prior to and 60 hr after GVG, nor in rats or mice shocked 12 hr prior to and 60 hr after GVG.

The maximal effect of GVG on chemical and electrical seizures in mice occurred during reported peak levels of whole brain GABA, consistent with previous reports regarding the time-course of GVG in genetic and kindled seizures. The failure of GVG to alter seizure threshold in doses which affect ECS and chemically-induced seizures suggest that it works via alteration of seizure spread. The ability of GVG to attenuate chemically-induced seizures and to exacerbate ECS is similar to our observations of ethosuximide, clinically effective in treatment of petit mal, and to clinical reports of exacerbation of grand mal seizures by ethosuximide.

287.11 THE ROLE OF SEROTONIN (5-HT) IN THE RESISTANCE OF "FLEXOR RATS" TO MAXIMAL ELECTROSHOCK-INDUCED HINDLIMB EXTENSION (HLE). R.A. Browning, J.K. Smith* and M.T. Brandon*. Southern Illinois Univ. School of Medicine, Carbondale, IL 62901.

Rats may be classified by maximal electroshock as either "flexors" (if they fail to exhibit HLE) or "extensors" (if they consistently exhibit HLE). Although only a small percentage (10-20%) of male Sprague-Dawley rats are true flexors, it has been suggested that these animals may serve as a naturally-occurring model for studying mechanisms of seizure attenuation (Buterbaugh, Life Sci. 23 2393, 1978). Since abolition of HLE in the maximal electroshock seizure (MES) test is widely used as an index of anticonvulsant drug activity, it is of interest to know more about the natural mechanisms that regulate HLE.

It appears that MES-induced HLE is very sensitive to manipulations in 5-HT. Thus, flexor rats can be converted to extensors by depletion of 5-HT, while extensors can be converted to flexors by treatments that increase 5-HT receptor activation (Buterbaugh Life Sci. 23 2393, 1978). Moreover it has been suggested that the resistance of flexor rats to HLE is due to increased whole brain levels of 5-HT (Buterbaugh, Neuropharmacology 16, 707, 1977). In order to test further this hypothesis and to gain a better understanding of where 5-HT might be acting to alter HLE, we examined 5-HT and 5-HIAA levels in 8 regions of the CNS, of age-matched flexor and extensor rats using high pressure liquid chromatography with electrochemical detection. Furthermore, we compared the in vivo synthesis rate of 5-HT between flexor and extensor rats in 6 regions of the brain, by measuring the accumulation of 5-HTP following aromatic amino acid decarboxylase inhibition with NSD-1015.

No differences in 5-HT or 5-HIAA levels were detected between flexor and extensor rats for any of the regions examined. Similarly, no differences in 5-HTP accumulation following decarboxylase inhibition were observed in the comparisons between flexor and extensor rats. These findings suggest that while pharmacological manipulations in 5-HT have marked effects on MES-induced HLE, neither an increase in 5-HT concentration nor 5-HT turnover can account for the natural resistance of flexor rats to HLE.

287.12 INITIATION OF PAROXYSMAL DEPOLARIZATION SHIFTS IN SINGLE CELLS OF THE SENSORIMOTOR CORTEX OF AWAKE CATS BY SCORPION VENOM (CENTRUROIDES SCULPTURATUS). N. Allon and C.D. Woody. Depts. of Anatomy and Psychiatry, UCLA Medical Center, Los Angeles, CA 90024.

The effects of intracellular injection of Centruroides sculpturatus (cs) venom were studied in 15 cells in the sensorimotor cortex of 8 awake cats. Techniques have been previously described (Woody and Black-Cleworth, J. Neurophysiol., 1973; Sakai et al, Neuropharmacol., 1979). Micropipettes containing 12.5 mg/ml of the venom in 2.25 M KCl were used as recording electrodes. Following 1-2 min of control recording, the venom was injected into the cells through the recording electrode using a pressure of 80-90 pounds/sq. in. An additional group of 10 cells recorded from 4 cats was injected with 2.25 M KCl only and used as a control. No significant differences were found between the groups prior to injection with respect to their resting potentials, spike heights or input resistances.

After injection of cs venom the following changes in the cells' characteristics were observed: 1) an increase in the cells' excitability 45-60 seconds after the injection of venom in comparison with their mean excitability prior to injection ($p<0.01$) and that of cells in the control group ($p<0.001$), 2) an increase in the cells' input resistance 45-60 seconds after injection in comparison with their mean resistance prior to injection and the resistances of cells in the control group ($p<0.02$ and $p<0.01$ respectively), 3) low frequency (0.2-1.0 Hz) fluctuations of 5-15 mV in membrane potentials of the neurons developing within 15-45 seconds after injection of scorpion venom but not after control injection of KCl. These fluctuations in membrane potential eventually gave way to paroxysmal depolarization shifts of similar appearance to those in epileptic cells. The changes were significantly different from those of cells in the control group injected with KCl only (X^2, $p<.001$).

Since cs venom acts by leaving the sodium channels in a partially opened state, we suggest that alterations in sodium channels may play an important role in initiating epileptiform activity of this kind. (Supp. by BNS 78-24146 and HD05958)

287.13 DECREASED MUSCARINIC RECEPTOR SITE AND CHOLINERGIC ENZYME ACTIVITY IN THE CENTRAL NERVOUS SYSTEM FOLLOWING EXPERIMENTAL FEBRILE CONVULSIONS IN THE DEVELOPING RAT. James A. McCaughran, Jr., Emmeline Edwards and Nisson Schechter. Long Island Research Institute, SUNY at Stony Brook, New York 11794

Experimental febrile convulsions (HC) induced by hyperthermia in the developing rat are a potentially useful animal model of human infantile febrile convulsions. In the present report, the effect that a series of HCs during development in the rat have on the ontogeny of the cholinergic system in selected brain areas was investigated.

Infant rats are subjected to a series of 10 HCs from 5-16 days of age. Control groups comprised rats that were exposed to a similar regimen of hyperthermia alone or were handled. At 2, 8, and 55 days after the last HC, the rats were killed and the activity of choline acetyltransferase (ChAT), acetylcholinesterase (AChE), as well as the concentration of nicotinic (aBuTX) and muscarinic (QNB) receptor sites, in the frontal cortex, hippocampus, hypothalamus, and cerebellum were investigated.

ChAT activity in the cerebellum and frontal cortex, but not the hypothalamus and hippocampus, was markedly affected by the series of HCs. The concentration of muscarinic sites in the cerebellum of the experimental group was similar to control at 2 days, greater than control at 8 days, and less than control at 55 days. Decreased muscarinic binding as well as a reduction in the activity of ChAT in the frontal cortex was also observed in the experimental group but only at 55 days after the last convulsion. Nicotinic receptor site concentration was similar in all groups.

The results of the present study clearly suggest that experimental febrile convulsions during development are able to elicit longterm biochemical changes in the central nervous system. The loss of cholinergic activity in the cerebellum and frontal cortex is consistent with a possible loss of cholinergic neurons. Because this effect is not apparent until 55 days after the last HC, the possibility that this reflects a secondary effect (e.g., transynaptic degeneration) rather than a primary effect must be considered. However, the fact that the differences were limited to the cerebellum and frontal cortex adds further support to previous studies which suggest that these two brain areas are particularly sensitive to the adverse effects of hyperpyrexia in both experimental animals and humans.

287.14 SYNCHRONIZING EFFECT OF EXTRACELLULAR ELECTRICAL FIELDS DURING SEIZURE-LIKE AFTERDISCHARGES IN RAT HIPPOCAMPAL SLICES. C.P. Taylor and F.E. Dudek. Dept. Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Although several mechanisms have been suggested for the paroxysmal depolarization shift of seizures, the synchrony of action potentials characteristic of epileptiform activity remains largely unexplored. Transverse (450 μm) slices of rat hippocampus were prepared conventionally. Chemical synapses were blocked with a physiological saline containing Mn^{2+} and lowered $[Ca^{2+}]$. Transmembrane voltage (E_m) was recorded differentially between two micropipettes, one intracellular in a CA1 pyramidal cell body and the second in the immediately adjacent extracellular space, allowing an accurate measure of E_m in the presence of large extracellular population spikes. The accuracy was evaluated by differential recording after the intracellular pipette was withdrawn.

Antidromic stimulation of the alveus at subthreshold intensity for a particular impaled neuron consistently revealed small depolarizations (DPs) which were only seen with differential recording. The DPs were of opposite polarity but similar waveform and related amplitude to extracellularly recorded population spikes, suggesting that they were caused by extracellular electrical fields (ephaptic interactions). Such DPs were finely graded in amplitude and could sum with depolarizing current, anode-break stimuli, or depolarizing afterpotentials to cause spikes. DPs could occur during the refractory period after a somatic spike and were unaltered by hyperpolarization. Thus DPs differ from electrical or chemical synaptic potentials or blocked axonal or dendritic spikes.

Prolonged (2-4 hr) incubation of slices in static physiological solution caused a gradual increase in excitability. In over 20 such preparations, prolonged afterdischarges (0.25-10 sec) of large population spikes (up to 20 mV) were elicited by a single alvear stimulus. Despite blockade of chemical synapses, both intracellular and extracellular recordings indicated pronounced synchrony of spikes during afterdischarges. When early intracellular action potentials of an afterdischarge were blocked by hyperpolarizing current, subsequent action potentials remained synchronized with population spikes, indicating an extrinsic synchronizing influence. Transmembrane intracellular records revealed that DPs similar to those described above occurred synchronously with each population spike and also appeared as prepotentials underlying somatic spikes.

Therefore, we suggest that transient electrical fields during population spikes cause membrane depolarizations which are important for synchrony of action potentials observed during epileptiform activity of the hippocampus.

Supported by NIH Postdoc. Fellowship (CT) and grant NS 16683.

287.15 SYNCHRONIZATION OF HIPPOCAMPAL CA 1 PYRAMIDS IN THE ABSENCE OF SYNAPTIC TRANSMISSION. H. L. Haas* and J. G. R. Jefferys* (SPON: ENA) Neurophysiology Lab., Neurochir. Univ.klinik, 8091 Zürich, Switzerland.

A synchronous and rhythmic bursting activity develops in the CA 1 neurone population in hippocampal slices of the rat after incubation in low Ca (0.2 mM), high Mg (4 mM) medium at 32°C for about 2 hours. No sign of synaptic transmission is found in this condition but alveus stimulation evokes a repetitive discharge of CA 1 pyramidal cells (5 - 20 population spikes) and often triggers a field burst (FB) similar to the spontaneous ones. FBs last 1 to 15 sec, recur every few seconds to minutes and persist with great regularity for many hours. Intracellular recording revealed depolarizing shifts without obvious changes in membrane resistance. Both the population spikes within the FBs and the FBs themselves are synchronous throughout the CA 1 region. Micro-lesion experiments and local TTX application showed that the synchronization occurs in the alveus where the axons of CA 1 pyramids are densely packed. There is no indication for a particular group of pacemaker cells. Blocking of ion pumps dramatically increases the burst frequency (ouabain 0.1 μM, lithium 1 - 5 mM, oxygen reduction). FBs were never observed in the CA 3, CA 4 and dentate regions (32 slices).

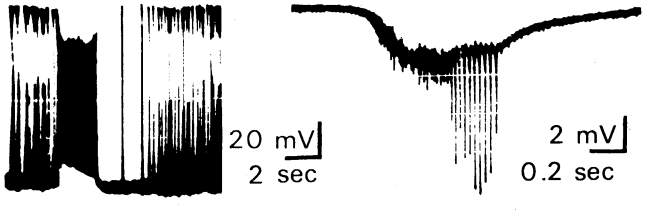


Fig: Spontaneous bursts in CA 1. Left: intracellular record, right: field burst (FB).

This novel paroxysmal activity could be important in brain pathophysiology and may provide new insights into normal and abnormal rhythmic firing patterns.

287.16 CA1 PYRAMIDAL CELLS EXHIBIT SPIKE FREQUENCY ADAPTATION AND A SLOW OUTWARD CURRENT. A. C. Bragdon* and W. A. Wilson (SPON: T. Slotkin). Depts. of Medicine and Pharmacology, Duke Univ. Medical Center and VA Medical Center, Durham, NC 27705.

Spike frequency adaptation (SFA) is defined as the ability of a neuron to slow its firing rate while depolarized by intracellular injection of a constant current. Understanding the basis of this intrinsic inhibitory process could improve our understanding of cellular mechanisms of epilepsy and anticonvulsant drug action: loss of this process may be involved in epileptogenesis; alternatively, drugs which enhance this process are potential anticonvulsants. In fact, studies of SFA in Aplysia neurons have shown that phenytoin and anticonvulsant barbiturates enhance SFA. The purpose of these experiments was to study this process in a convenient mammalian preparation.

Using the in vitro rat hippocampal slice preparation, CA1 pyramidal cells were studied intracellularly with KAc and KCl electrodes. To study SFA, spiking was recorded during a series of 4-sec constant current depolarizations. To study the currents underlying SFA a single electrode voltage clamp (VC) system was used. Cells were kept at a holding potential (HP) near resting potential. Currents were recorded during and after a series of 4-sec constant voltage depolarizations.

SFA: In all cells studied, a 4-sec constant current depolarization evoked this sequence: a 300-400 msec phase of brisk initial firing during which spike frequency declined rapidly (fast SFA), followed by a phase of slower firing during which spike frequency declined more slowly (slow SFA) or remained constant.

VC studies: During a 4-sec constant voltage depolarization, all cells developed a persistent net outward current (I_o) in two phases which paralleled the two phases of SFA: I_o rose rapidly in the first 400 msec, then continued to rise more slowly or remained constant. Following return to HP, outward tail currents were observed. These were enhanced by shifting the HP to a more depolarized potential and eliminated with HPs of about -95 mV.

In a few cells, the fast SFA phase was followed by a silent period (~ 500 msec) before slower firing began. In VC studies of these cells, I_o first rose rapidly, then exhibited an even higher peak before falling to continue with the usual slowly rising phase. The time course of this extra hump in I_o coincided with the silent period during SFA.

These studies demonstrate (1) CA1 pyramidal cells exhibit SFA, and (2) the mechanism underlying SFA appears to involve a slowly developing outward current, the strength of which correlates inversely with firing frequency. Studies of the ionic basis of this process and the effect of anticonvulsant drugs on it should enhance our understanding of and treatment of epilepsy.

Supported by VA Medical Center, Durham, NC.

287.17 INTRACELLULAR AND FIELD POTENTIAL ANALYSIS OF A SLOW POTENTIAL ASSOCIATED WITH PENICILLIN-INDUCED EPILEPTOGENESIS IN IMMATURE CA3 HIPPOCAMPAL PYRAMIDAL CELLS. Robert J. Brady* and John W. Swann (SPON: M. Pierson). Lab. of Developmental Neurophysiology, BDI, CTR. for Labs and Res. NYS Dept. of Health, Albany, NY 12201

When hippocampal slices, taken from 9-19 day old rat pups, are exposed to a bathing medium containing penicillin (1.7 mM final concentration), the CA3 pyramidal cells generate bursts of population spikes similar to those recorded extracellularly from mature CA3 pyramidal cells. In contrast, however, these epileptiform bursts in the immature slices are invariably followed by a large slow negative field potential (recorded in the pyramidal cell body layer). Riding on the envelope of this negative field is an afterdischarge with a duration which varies directly with that of the slow field potential. The slow potential is often 30 sec in duration. Intracellular recordings reveal that, following the paroxysmal depolarization shift (PDS) and therefore coincident with the slow field potential, individual CA3 pyramidal cells generate a slow depolarizing afterpotential. This is in sharp contrast to the afterhyperpolarization seen in the mature CA3 neurons.

The slow negative field potential is associated with the ability of the immature slices to undergo prolonged afterdischarges. Therefore, we have conducted experiments which have systematically examined the laminar distribution of this field potential. Under direct visual control and with the aid of an eyepiece micrometer, field potential recordings were made in 50μ steps across stratum radiatum and oriens. A second microelectrode, placed and maintained in the center of the cell body layer throughout the course of the experiment, monitored any systematic alteration in the field potentials. Slow potentials less than 500 msec in duration were selected for analysis. Our recordings demonstrate that the slow negative field potential recorded in the cell body layer: 1. has a peak negativity 25-75μ from the edge of the cell body layer in oriens; 2. is negative throughout oriens, 3. is positive throughout radiatum (except for the 150μ closest to the cell body layer where is is small in amplitude). This laminar distribution stands in sharp contrast to that of the epileptiform burst since the envelope on which the burst of population spikes rides, has two peaks of large negativity; one approximately 250μ from the cell body layer in radiatum, the other 150μ out in oriens. The burst is positive in the cell body layer and in the more distal portions of radiatum. These data suggest that in immature pyramidal cells the PDS may originate in both dendritic trees. In addition they dramatically illustrate that the slow field potential associated with afterdischarge generation is of a different origin than the epileptiform bursts. (Supported by NIH Grant NS-18309)

287.18 A BASILAR DENDRITIC SLOW POTENTIAL AND AFTERDISCHARGE GENERATION IN IMMATURE CA3 HIPPOCAMPAL PYRAMIDAL CELLS. John W. Swann and Robert J. Brady*. Lab. of Dev. Neurophysiology, BDI, Ctr. for Labs and Res. NYS Dept. of Health, Albany, NY 12201.

In a previous study our laboratory described the unusual ability of the CA3 region of immature hippocampal slices (taken from rat pups 1-2 weeks of age) to undergo prolonged epileptiform afterdischarges when exposed to penicillin (Swann, Soc. Neurosci. Abst. 7:589, 1981). Associated with these afterdischarges is a slow negative field potential which can be recorded in the pyramidal cell body layer. Recent experiments have shown that this field has a peak negativity in stratum oriens 25 to 75 μM from the edge of the cell body layer. As a first step towards understanding the origin of this slow field we attempted to elicit such a potential in normal bathing medium by electrical stimulation. We have found that a slow field potential can be routinely recorded in the immature CA3 region in response to a single stimulus (50-100μV-100μsec) applied to stratum oriens or radiatum. These fields are often several millivolts in amplitude and approach a duration of 1 sec. Moreover, oriens stimulation is by far the more effective. As with the penicillin associated slow potential this slow potential has a peak negativity just below the pyramidal cell body layer in oriens, even when the stimulating electrode is placed in the most distal aspects of this basilar dendritic layer. The field epsp, recorded in oriens, has a distinctly different laminar field potential distribution. With repetitive stimulation (1-5 Hz) the slow negative potential temporally summates to produce a large steady field. In addition it shows dramatic frequency potentiation during the early phase of such trains. Following such repetitive stimulation this slow potential is always dramatically potentiated (in some cases 400%) for 5-15 sec. In addition, the negative field often outlasts the stimulus train and afterdischarges of 15-30 sec develop, which ride on the envelope of this field.

Intracellular recordings have been made from the CA3 cell body layer, simultaneous with the extracellular events reported above. These demonstrate that, coincident with the slow negative field, individual pyramidal cells undergo a large slow depolarization. As observed for the slow field potential these depolarizing potentials are greatly potentiated by repetitive orthodromic stimulation and can lead directly to an intense tonic depolarization observed during afterdischarge generation. (Supported by NIH Grant NS-18309)

287.19 PRESSURE INCREASES EXCITABILITY OF PYRAMIDAL CELLS IN HIPPOCAMPAL SLICES MAINTAINED IN VITRO. P.G. Kaufmann, C.R. Miller*, P.B. Bennett and R. Baker*. F.G. Hall Environmental Laboratory, Duke Univ. Med. Ctr., Durham, NC 27710.

Elevated hydrostatic or gas pressures above 30 atmospheres cause functional changes in various excitable tissues as well as in a variety of species. Although excitatory effects on the CNS of intact animals are well documented (Brauer, R.W., J. Appl. Physiol., 37:844, 1974) several studies on invertebrate in vitro preparations have reported diminished synaptic transmission (Campenot, Comp. Biochem. Physiol. 52:133, 1975). The in vitro rat hippocampal slice preparation affords a technique by which pressure effects on mammalian CNS tissue can be assessed directly without the influences of any respiratory, cardiovascular, or other changes which might be occurring simultaneously.

Extracellular recordings were made from rat hippocampal slices exposed to pressures up to 60 atmospheres of helium. Electrical stimulation (20 μsec, 0.5-10 mA) of the stratum radiatum (SR) evoked field EPSP's which were recorded by a microelectrode in the SR. Population spikes in the CA1 region of the stratum pyramidale were recorded by another microelectrode. Input-output functions were plotted between the compound presynaptic spike and the compound EPSP, and between the rising slope of the EPSP and the amplitude of the compound postsynaptic spike in CA1. Compared with control recordings, elevated helium pressures did not consistently alter the amplitudes of EPSPs for given amplitudes of presynaptic spikes. The amplitude of compound postsynaptic spike, on the other hand, was enhanced for a given EPSP. These effects were more pronounced at 60 atmospheres than 30 atmospheres, and showed some reversibility with partial decompression. Our results indicate that although high pressure does not significantly alter synaptic transmission in this preparation, excitability of hippocampal pyramidal neurons is increased. Supported by ONR N00014-75-C-0553.

287.20 SINGLE UNIT STUDY OF TRANSITION FROM SPINDLES TO SPIKE AND WAVE DISCHARGES (SW) OF FELINE GENERALIZED PENICILLIN EPILEPLSY (FGPE) USING SIMULTANEOUS RECORDINGS FROM THALAMUS AND CORTEX. R.S. McLachlan*, M. Avoli, P. Gloor and G. Kostopoulos. Montreal Neurological Institute & Dept. of Neurology & Neurosurgery, Montréal, Canada, H3A 2B4.

Previous data had suggested that SW of FGPE result from an increased excitability of cortical neurons to thalamocortical volleys which would normally produce spindles. Furthermore, unit recordings in thalamus (n.lateralis posterior-pulvinar) during fully developed SW have shown that most thalamic neurons fire in bursts which immediately (5-45msec) precede the cortical neuronal bursts associated with the EEG "spike" of cortical SW. To explore this further, the transition from spontaneous spindles to generalized SW following i.m. penicillin (350,000 I.U./kg) was studied in both cortex (areas 5 and 7) and thalamus (n.lateralis posterior-pulvinar). Data were statistically analyzed by a computer.

Findings in the cortex were as previously described; namely, gradual increase in unit firing probability during a spindle wave as it was transformed into the "spike", while a period of silence was associated with the emerging "wave" of SW. Similar changes occurred in the thalamus either concurrently with or shortly after the onset of the changes in the cortex. Although during the transition the overall firing rate of cortical units gradually increased, thalamic unit activity first decreased in many cases during early development of SW and then increased. In some of these experiments where the thalamic changes followed those in the cortex, during the lag period, thalamic neurons fired at the spindle frequency while cortical neurons were firing at the lower SW frequency. However, during fully developed SW discharges thalamic neurons, as previously reported, displayed a phasic increase of firing probability whose peak preceded that of the cortical neurons. In two cases the thalamic firing followed the cortical burst. The cross-correlation between cortical and thalamic unit activity was low during spindles but gradually increased during the development of SW.

The data further support the hypothesis that generalized SW develops as a result of an abnormal cortical response to normal thalamic inputs. In addition, they show that thalamic neurons, as SW emerge, are entrained by the cortex to produce synchronized activity which in turn can contribute to sustain the SW rhythm.

287.21 CELLULAR MECHANISM OF SYNCHRONIZED BURSTING DURING INTERICTAL SPIKES. R.D. Traub and R.K.S. Wong. IBM Watson Res. Ctr., Yorktown Heights, NY 10598 and Univ. Texas Med. Branch, Galveston, TX 77550.

Synchronization of cellular bursting is the fundamental issue in understanding interictal spikes in the disinhibited hippocampal slice. We have shown (Traub and Wong, J. Neurophysiol., in press) that 3 experimentally observed features of the system underlie this phenomenon: Intrinisc bursting, disinhibition, and mutual excitation. A 10x10 network of simplified model neurons exciting one another via sparse random interconnections reproduces important features of interictal spikes: the field potential, the long latency from a localized stimulus to onset of the event, and periodicity of interictal spiking. Using the same network (with probability of one cell connecting to any other cell 5%) but with each cell described by a detailed comparmental model, we are now able to reproduce intracellular records as well: typical bursting in most cells with latencies of tens of ms and double bursts in other cells (Traub and Wong, Science, in press). Passing a hyperpolarizing current through a single cell in the network reveals the underlying excitatory synaptic input from other cells that elicits intrinsic bursting, just as occurs experimentally. Some synchronization occurs with connection probabilities as low as 2.5%. Including electrotonic junctions in the network caused complex effects (e.g. prolonging the latency of an interictal spike), the nature of which depended on the strength and density of chemical synapses. Simulations were done of a 3x3 network, in which, in addition to random mutual excitation, each cell inhibited all the others. Depending on the strength of inhibition, one might see either no synchroinzation, partial interictal spikes (where only some cells were recruited into the event) or full interictal spikes. If the intrinsic AHP conductance is decreased, some cells go into depolarization block and in turn excite other cells into depolarization block. The same principles which explain synchronous bursting may also explain the development of certain seizure phenomena.

287.22 SEIZURE GENERATION IN HIPPOCAMPAL SLICES FROM IMMATURE RABBIT. P. A. Schwartzkroin and M. M. Haglund*, Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

Studies of epileptogenic mechanisms in the *in vitro* hippocampal slice preparation have focused on interictal burst generation. These experiments have not been extended to ictal, or seizure, episodes since seizures are rarely observed in healthy hippocampal slices. In the course of our recent studies of rabbit hippocampal development, we found that spontaneous or stimulus-evoked seizure episodes occurred regularly in tissue from 9-12 day old pups.

Intracellular and field potential recordings were made in the CA1 and CA3 regions of hippocampal slices. Intracellularly, the following sequence of events characterized the seizure episodes: A flurry of synaptic events caused a small depolarization of the cell membrane which was followed (at a quite variable delay) by an abrupt cell depolarization of 30-50 mV. In most cases, the cell discharged a few action potentials during the depolarization onset, and then was silent. Cells remained depolarized, with large conductance shunts, for 30-80 seconds; they then started a gradual repolarization, accompanied by burst discharges, and returned to normal resting levels (or a slightly hyperpolarized level) within 30-60 seconds. These episodes could occur, without any obvious precipitating cause, every 10-20 minutes for 4-5 hrs.

Simultaneous extracellular recordings, with an electrode located within 50 μ of the intracellular electrode in stratum pyramidale, revealed dramatic negative field potential shifts (20-50 mV) correlated with the intracellular changes. Return of the extracellular dc potential toward its pre-seizure level occurred as burst discharges became evident in the cells. These bursts were reflected in field potential population spikes, and suggested that a synchronized population was involved in this clonic discharge. Simultaneous intracellular recordings from two pyramidal cells showed that although these afterdischarge phenomena were well synchronized, the events during seizure onset could differ both in timing and the rate of depolarization.

These seizure-like events are not normally seen or elicited in healthy mature hippocampal slices, nor are they observed at earlier stages of development. Their repetitive, spontaneous occurrence in tissue with otherwise normal properties argues against interpretation of these events as simply signs of deteriorating slices. Similar events have been elicited in mature hippocampal slices by lowering bath chloride concentration. Given the demonstrated immaturity of inhibitory PSP efficacy in immature tissue, it seems likely that seizure genesis depends on chloride conductance in immature neurons. We believe that these phenomena now provide us with a useful *in vitro* model of ictal activity.

(Supported by NINCDS grants NS 15317, 04053 and 00413)

287.23 EXPLOITATION OF INTERLAMINAR CIRCUITRY BY EVOLVING EPILEPTIC FOCI IN NEOCORTEX. J. S. Ebersole and A. B. Chatt. Epilepsy Center, V.A. Medical Center, West Haven, CT 06516 and Department of Neurology, Yale University School of Medicine, New Haven, CT 06510.

Intra- and interlaminar circuitry in neocortex provides the substrate for serial cortical processing of information by neuronal populations. The exploitation of this existing connectivity by epileptic foci during their evolution can be appreciated by viewing epileptogenesis simultaneously from multiple laminar perspectives. Microelectrodes with three recording barrels, which have longitudinal tip separations, were positioned to span the layers of cat striate cortex. Regional field potentials and multi-unit activity were evoked by visual field specific photic stimulation. Injection of sodium penicillin (40mM) in nanoliter volumes, through a twin barrel at one of the three recording levels, induced discrete epileptic foci which demonstrated a characteristic progression of response abnormalities.

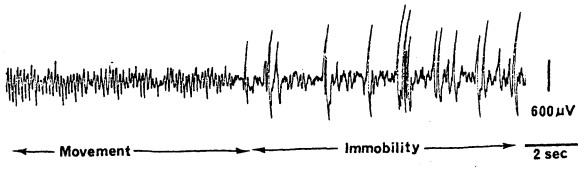
Interlaminar "projection" of longer latency epileptiform activity (late responses, LR) tended to follow anatomically recognized pathways. Late responses induced in layer 4 were reflected nearly synchronously in layers 2-3, both in the form of a field potential and multi-cellular burst. The major outflow from layer 4 is to these superficial laminae. In most experiments LR's in layer 4 were elicited so quickly (15-30 sec) that penicillin diffusion into the superficial laminae was an unlikely cause for counterpart LR's there. Negative field potentials and cellular bursts were recorded in layers 5-6 and 4 following penicillin microinjection and the evolution of LR's in layers 2-3. The response from deep cortex was often more prominent, since this region receives collaterals from superficial pyramidal axons exiting the cortex. LR's from foci in layers 5-6 appeared to evoke counterpart potentials and unit bursts in layer 4, probably by way of axon collateral pathways that have been demonstrated from layer 6 pyramidal to layer 4 stellate cells. In both latter situations, however, development of the focus was protracted and appeared to depend upon the diffusion of penicillin into lamina 4 to induce an enhanced primary latency response there. The resultant increased outflow from layer 4 could provide the other laminae with needed pacing.

Layer 4 is not only more susceptibile to epileptogenesis, but appears to influence the expression of epileptic response abnormalities in other layers.

287.24 SPONTANEOUS EEG SPIKES IN THE HIPPOCAMPUS OF NORMAL BEHAVING RAT. Shinya S. Suzuki* and Grant K. Smith. Dept. Psychol., McMaster Univ., Hamilton, Ontario, Canada L8S 4K1.

Spontaneous fast (unit) and slow (EEG) potentials were recorded simultaneously from the CA1 region of the dorsal hippocampus by means of a movable microelectrode in normal behaving rats. Large amplitude (~3mV) negative slow potentials of 40-80 ms. duration (EEG spikes) with frequencies in the range 0.1-5 Hz were consistently recorded from the middle apical dendritic layer (stratum radiatum) during awake immobility, automatic movement, and slow wave sleep (see figure). Rhythmical slow EEG activity (RSA or theta) predominated during voluntary movement and paradoxical sleep. Laminar analysis indicated that the spikes were positive in stratum oriens and changed polarity in the vicinity of stratum pyramidale. The voltage gradient across the polarity reversal zone was sharp and reached about 2 mV/50 μM. Negative spikes were generally larger than positive spikes and had a sharper and more localized peak in their depth profile. Peak negativity occurred at about 200 μM below the reversal point. The EEG spikes were usually accompanied by synchronous action potential bursts in the pyramidal cell layer. These bursts were absent during behaviours associated with RSA.

These and other observations suggest that the EEG spike represents a focal and synchronous excitation of the middle dendritic layer which triggers action potential bursts in numerous pyramidal cells. Available anatomical and evoked field potential evidence indicates that the Schaffer collateral and commissural fibers terminating on the middle dendritic tree may be responsible for this synchronous excitation. (Supported by Grants from NSERC and by scholarships from the Canada Council and SSHRC.)



287.25 VALPROIC ACID DOES NOT ALTER CEREBRAL OR HEPATIC ZINC METABOLISM, E.J. Kasarskis, D. Walls*. Dept. Neurology, VA and Univ. Ky. Med. Ctrs. and Sanders-Brown Aging Ctr., Lexington, Ky. 40536

The similiarity between the side effects of valproic acid (VPA) and zinc (Zn) deficiency have been noted by Hurd et al. (Soc. Neurosci. Abstr. 7:813, 1981) who, in addition, provided preliminary evidence that VPA binds Zn in vitro. This study investigated the effect of VPA on Zn metabolism in vivo in rats fed Zn-adequate diets.

VPA (in 0.9% NaCl, pH 7.4)was administered to adult Sprague-Dawley rats fed Zn-adequate commercial chow and water ad libitum according to 2 schedules: (A) 100 mg /Kg BW, SQ daily for 7 days ("chronic therapeutic") or (B) 500 mg/Kg Bw SQ as a single dose ("acute toxic"). Control rats received an equal volume of saline SQ; body weight and food consumption were not altered by VPA treatment. Rats treated with "chronic therapeutic" VPA attained blood levels between 30-60 mcg/ml.

Following VPA dosing, rats were briefly anesthetized with methoxyflurane while a jugular cannula was placed for intravenous administration of $^{65}ZnCl_2$ (25 μCi/100g BW.) After 6 hrs samples of liver, plasma, muscle, kidney, CSF, brain (divided into 10 regions), and sciatic nerve were obtained, weighed, and assayed for ^{65}Zn content as described previously (Soc. Neurosci. Abstr. 7:87,1981). Though Zn uptake into brain proceeds slowly over several days, the maximum accumulation in liver occurs between 4-6 hrs after administration of a single ^{65}Zn dose (Soc. Neurosci. Abstr.7:87, 1981). Therefore, sampling at 6 hrs measures predominately the net rate of ^{65}Zn brain uptake but would be sensitive to changes in uptake, turnover, or both in liver. Chronic administration of VPA in therapeutic doses did not alter ^{65}Zn metabolism in any tissue sampled. For example, the ^{65}Zn content of hippocampus was 19,700 $\pm$ 400 cpm/g dry weight and 20,600 $\pm$ 500 cpm/g dry weight in VPA and control animals, respectively. Rats receiving a single acute toxic dose of VPA had higher plasma levels of ^{65}Zn suggesting that plasma clearance may be impaired. However, the ^{65}Zn content of brain and other tissues sampled was not significantly altered by VPA treatment.

We conclude that VPA in the doses employed does not adversely affect Zn metabolism in brain or liver of normal rats. However, our studies do not eliminate the possibility that VPA may interfere with Zn metabolism either in Zn-deficient animals or after chronic administration of VPA in toxic doses. (Supported by VA Research Grant; VPA was a gift of Abbott Laboratories).

287.26 RAPID KINDLING IN THE HIPPOCAMPUS. C.F. Zorumski, E.W. Lothman and J.M. Hatlelid, Depts. Psychiatry, Neurology and Pharmacology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Previous reports have shown that an electrical stimulus, delivered once daily to the amygdala, leads to an intensification of seizures, i.e., kindling. We were interested in whether seizures arising in the hippocampus at much shorter intervals had similar consequences. A bipolar electrode was stereotactically implanted in the ventral hippocampus of albino rats and connected to an electronic switch that allowed stimulation and recording from the same site. A standard stimulus (100-1 msec, 400 µA, biphasic pulses at 10 Hz.) was delivered every 5 minutes for 6 hours, withheld 12-18 hours and then reinstituted. This current intensity was 3-5x afterdischarge threshold in individual animals. Lengths of afterdischarges (AD) were measured on an oscilloscope and EEG chart recorder and coincident behavior scored as: 0 = no change; 1 = wet dog shakes; 2 = head nod; 3 = limb clonus; 4 = rear; 5 = rear and fall. Initial stimulations resulted in short AD and mild seizures (0-2). Subsequently, prolonged AD and severe seizures (3-5) appeared within 2-3 hours. Following severe seizures on the first day of stimulation, 20-60 minutes elapsed before another severe seizure occurred. However, during this period each stimulus provoked mild seizures. During the second day of stimulation, severe seizures were seen from the outset and the interval between severe seizures was shortened. This phenomenon persisted for up to three weeks, the longest period studied. If the interstimulus interval was lengthened to 30 minutes on the second day, severe seizures were produced with each stimulus. After the first day of stimulation, trains of various lengths and intratrain frequencies trigggered severe seizures if AD were produced. In some animals bipolar electrodes were placed in other limbic areas (contralateral hippocampus, amygdala and nucleus accumbens). In naive animals stimulation of these sites produced short AD and only mild seizures. In contrast, after completion of the standard stimulation paradigm described above, the same stimuli caused severe seizures. We conclude that rapidly occurring hippocampal seizures have several results. The appearance of severe seizures even though the inciting stimulus is not altered, the long lasting nature of this change, and the ability to provoke severe seizures from other sites all suggest that processes akin to classical kindling are activated. In addition there is an inhibitory process, following severe seizures, that sets the interval before another severe seizure can occur. The mechanism for the shortening of this interval on the second day of stimulation is not known but could relate to a maturation of the kindling process, a resolution of inhibition, or both.

287.27 ELECTROPHYSIOLOGIC AND METABOLIC MAPPING OF HIPPOCAMPAL SEIZURES. J.M. Hatlelid, E.W. Lothman and C.F. Zorumski. (SPON: J.L. Trotter). Depts. Pharmacology, Neurology, and Psychiatry, Washington Univ. Sch. Med., St. Louis, MO 63110.

In order to study how seizures spread from the hippocampus we used multiple depth recordings and ^{14}C-2-deoxyglucose (DG) autoradiography. Seizures were produced with electrical stimulation (100-1 msec, 400 µA biphasic pulses at 10 Hz.) every 5 minutes through a bipolar electrode stereotactically positioned in the ventral hippocampus of albino rats. As described elsewhere (these abstracts), this paradigm initially caused short afterdischarges (AD) and mild behavioral seizures; later there were prolonged AD severe behavioral convulsions. Bipolar electrodes were placed in the nucleus accumbens (NA), globus pallidus (GP), amygdala (A), entorhinal cortex (EC), substantia nigra (SN), caudate-putamen (CP), and hippocampus at the site of stimulation (I-HC) and contralaterally (C-HC). Monopolar electrodes were inserted in the frontal (F) and parietal (P) skull. Recordings from these electrodes showed that all of the provoked seizures were accompanied by synchronized epileptiform discharges in I-HC, C-HC, A, EC, NA, and SN. Only with severe seizures during which there was limb clonus was there spread of electrical seizure activity into the GP, CP and FP channels. DG autoradiography obtained during mild seizures showed increased metabolism in both hippocampi; those obtained during severe seizures showed increased metabolism in the hippocampi, medial and lateral septum, amygdala, substantia nigra and nucleus accumbens.

We conclude that there are structures to which seizures preferentially spread from the hippcampus, including areas outside the classical limbic system, and that specific structures can be linked to certain behavioral features of "limbic seizures". An unexpected result was the lack of increased glucose metabolism during mild seizures in extrahippocampal structures that displayed seizure activity with EEG recordings. The reason for this is not clear, but may relate to the fact that EEG recordings delineate several physiological processes that occur with time courses of seconds while DG autoradiography integrates the total metabolic response over 45 minutes.

287.28 EFFECTS OF CONVULSANTS ON THE SENSORY RESPONSES OF NEURONS IN AMYGDALA AND BRAIN STEM RETICULAR FORMATION. C.L. Faingold, W.E. Hoffmann* and D.M. Caspary. Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708.

Sensory stimuli can initiate generalized seizures in animals treated with convulsants. Subconvulsant doses of pentylenetetrazol (PTZ), strychnine (ST), bemegride (BMG) and bicuculline (BIC) extensively enhance the sensory responses of the vast majority of brain stem reticular formation (RF) neurons, but the responses of neurons in certain other brain regions are affected to a minor degree by these agents (Faingold, Prog. NeuroPsychopharm., 2:401, 1978; Faingold and Hoffmann, Neurosci. Abs., 7:812, 1981a; Faingold and Hoffmann, Electroenceph. clin. Neurophysiol., 52:316, 1981b). Previous studies have shown that the amygdala is a most sensitive brain area to seizure induction by local convulsant application and electrical stimulation. The present study examined 1) the effects of these convulsants on neuronal responses in the RF in comparison to simultaneous effects in the amygdala and 2) the effects of sequential administration of two different convulsants on the responses of the same neuron.

The effect of infusion of PTZ, ST, BIC, BMG and physostigmine at rates of 5, 0.025, 0.025, 1.8 and 5 mg/kg/min i.v., respectively were examined in locally anesthetized paralyzed or spinal cord-transected (C-1) respired cats. Neuronal responses to visual, auditory, somatosensory and/or vibrissa manipulation in mesencephalic reticular formation (MRF) and simultaneously recorded amygdala neurons were analyzed using post-stimulus time histograms. Overall, the sensory responsiveness of 90% of MRF and 55% of amygdala neurons was enhanced following convulsant administration. The degree of enhancement in MRF exceeded that in amygdala in cases where simultaneously recorded neurons responded to the same stimulus. In ten MRF neurons which recovered from convulsant-induced response enhancement a second convulsant with a different presumed mechanism of action was administered. Since enhancement was also observed with the second agent although it presumably affects a different transmitter, this suggests that response enhancement may not be transmitter specific. This finding is consistent with the report of Pellmar and Wilson (Science, 197:912, 1977) which indicates that many convulsants have a common effect beyond the level of the receptor, on the chloride conductance mechanism (ionophore).

Since sensory stimuli can trigger seizures after subconvulsant doses of these convulsants, the degree of response enhancement in a brain region may reflect the relative involvement of that region in seizure initiation. If so, amygdala, lateral geniculate, hippocampus and inferior colliculus neurons may not play major roles in seizure initiation (Faingold and Stittsworth, Electroenceph. clin. Neurophysiol., 49:168, 1980; Faingold and Hoffman 1981a; preliminary findings). By contrast, pericruciate cortex and especially brain stem reticular formation neurons are extensively enhanced (Faingold and Hoffmann, 1981a) suggesting that these regions may play an important role in the convulsant-induced sensory initiation of seizures. (Supported in part by CRC funds, SIU Foundation and NIH Grant NS 13849).

287.29 NEURONAL STRUCTURE AND ACTIVITY IN A FOCAL MOTOR SEIZURE MODEL, R. S. Greenwood, S. E. Godar* and K. K. Winstead* Dept. of Neurol. and Neurobiology Program. U.N.C. Sch. of Med., Chapel Hill, N.C.

A focal penicillin seizure model was used to study the relationship between motor behavior, neuronal activity and cellular anatomy. A stable penicillin seizure focus was created in the pericruciate cortex of pentobarbital anesthetized cats by injection of penicillin (500 units) into the cortex. In extracellular recordings a negative interictal spike (IS) developed immediately at the site of injection while a positive IS spike appeared in the cortex around the focus. When the injection site was made in the same location in each animal (area 4 near the posterior end of the presylvian sulcus) a muscle twitch in the contralateral shoulder reliably resulted. Intracellular recordings from the center of the focus revealed the previously-reported paroxysmal depolarizing shift (PDS). In the area surrounding the focus inhibition predominated in intracellular recordings. This zone of inhibition could extend up to several millimeters away from the center of the focus.

Intraneuronal injection of Lucifer Yellow revealed that most cells, regardless of location, were either pyramidal cells or were located in layers III and V of the cortex. No correlation was found between cell type and behavior during an interictal spike. Neurons injected in the focus, however, were less likely to have extensive processes and often had dendritic irregularities. No dye-coupling of cells was observed.

Cells fulfilling the physiologic criteria of glial cells were encountered. During the IS these cells had depolarizing responses and slow repolarization. When filled with Lucifer Yellow, these cells appear amorphous and are without processes.

We conclude that the PDS behavior of neurons in a penicillin focus in motor cortex produces focal motor activity corresponding to that occurring during electrical stimulation of the same area. PDS neurons are more likely than inhibited neurons to have partially filled processes when filled with Lucifer Yellow.

This work supported in part by USPHS General Research Support Award 5-S01-FR-05406.

287.30 CONVULSANT BURSTING RESULTING FROM CHANGES IN CURRENTS CHARACTERISTIC OF NON-BURSTING NEURONS. J.C.Fowler and L.D.Partridge. Dept. of Physiol., Univ. of N.M., Albuquerque, N.M., 87131

The convulsant drug Pentylenetetrazol (PTZ) induces epileptiform bursting in normally non-bursting molluscan neurons. The end stage burst behavior is the development of plateaus characterized by a rapid onset. This rapid onset of depolarizing shift suggests a possible triggering role of the inward spike currents.

We used voltage clamp pulses to investigate the effect of PTZ (20-60 mM) on the Hodgkin-Huxley type parameters of the two rapidly activating inward current components associated with the spikes. These two currents can be distinguished by their steady state and kinetic properties.

In neurons of the pond snail, Lymnaea stagnalis the Na current rapidly activates and inactivates while the Ca current (as observed in Ba substituted TEA solution) rapidly activates but only partially inactivates.

PTZ effects both of these currents similarly causing a decrease in peak conductance and leftward shift of both activation and inactivation curves with no change in slope. There is also an increase in activation and inactivation time constants as measured from non-linear least square fits of current traces.

The decrease in peak conductance would seem to favor a decrease in excitability. However, two other changes result in an overall increase in excitability. First, the leftward shift of the activation curve, estimated from non-linear least square fit of peak current I-V curve, results in a reduction in spike threshold. Secondly, opposing K currents are concomitantly reduced in the presence of PTZ.

The development of depolarizing plateaus suggests the presence of a slowly inactivating or non-inactivating depolarizing current. Computer simulation demonstrates that the non-inactivating calcium current could satisfy this requirement. However, we have confirmed the observation made by others that PTZ-induced bursting persists in the absence of Ca but not in the absence of Na.

Current measurements at the end of 400 msec voltage clamp pulses in 0 Ca, TEA containing Ringers reveals the presence of small inward going rectification between -50 and -20 mV. This rectification is apparent in the presence and absence of PTZ. We suggest that this persistent inward current in the presence of otherwise PTZ-reduced currents assumes a role in the onset and maintenance of PTZ induced depolarization shifts.

288.1 THE STELLATE NEURONS IN LAYER IV OF PRIMARY AUDITORY CORTEX (AI) OF THE CAT: A STUDY OF COLUMNAR ORGANIZATION. Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley CA 94720.

The neurons in layer IV were studied as part of a larger inquiry into the organization of AI. Golgi, Nissl, and other material, and tissue for electron microscopy, was taken from the convexity of the middle ectosylvian gyrus, between the ectosylvian sulci. Layer IV is a slender strip, 200-250 µm thick, wedged between the pyramidal cells of layers III and V. Layer IV is readily distinguished, on connectional and architectonic grounds, from layers III and V since commissural neurons are common in these layers and rare in layer IV. The dendrites of layer IV neurons are stellate and occupy slab-like domains; the apical and basal dendritic arbors run at right angles to the pia. This confers a vertical appearance to layer IV and is reinforced by the largely radial orientation of stellate cell axons, and by the thick axonal endings which may be of thalamic origin. Layer IV cells form small clusters between zones of neuropil. The round or slightly oblate soma has a mean area of 158.0 sq. µm (s.d.: 53.8 sq. µm; range: 30.7-347.3 sq. µm; N=310; toluidine blue-stained, plastic-embedded, 0.5-2.0 µm thick sections). In Golgi material, small, medium, and large stellate neurons, each differing in dendritic structure and location in layer IV, are present. Small cells with 50-100 µm wide dendritic fields dominate the upper half of layer IV (IVa), where their apical dendrites, and the wider, basal arbors of deep-lying, layer IIIb pyramidal cells, mingle. The stellate cell axon also ramifies here. The medium-sized stellate neurons occur in both tiers of layer IV, while the large cells usually lie in layer IVb. The axons of medium and large stellate neurons ramify in layer IV and layer III--particularly in layer IIIb and, on occasion, layer II. These axons form narrow, vertical palisades with few side branches. Stellate cell dendrites are either smooth, sparsely-spined, or spiny. Their dendritic branching patterns and the distribution of their axons may vary. Other, "double-bouquet" cells send axonal and dendritic branches through layers III and Va. Layer IIIb pyramidal cell axons form extensive, vertical arbors in layer IV, and some branches descend and re-enter layer IV laterally. The clusters of layer IV stellate neurons, the vertical polarization of the axonal and dendritic branches, the radial distribution of intracortical and extrinsic axons, each, fortifies the columnar organization of layer IV.

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288.2 AXONAL AND DENDRITIC DOMAINS OF NEURONS IN CAT PRIMARY AUDITORY CORTEX (AI) RELATED TO BEST FREQUENCY MAPS. R. A. Reale*, J. Z. Feng* and J. F. Brugge* (SPON: R. Wenthold) Dept. of Neurophysiology and Waisman Ctr., Univ. Wisconsin, Madison, WI 53706.

We combined microelectrode-mapping and HRP histochemistry to study relationships between best frequency maps and patterns of anterograde and retrograde labeling of neuronal elements within AI of cat. Isofrequency lines were determined by microelectrode mapping of best frequencies to guide the iontophoretic injection of HRP. Frozen sections were cut parallel to the flattened cortical surface and reacted using a cobalt chloride modification of the diaminobenzidine protocol which produced Golgi-like staining of axons, dendrities and neuronal perikarya. All labeled neuronal elements in the vicinity of the injection zone were traced (Mag. 300X) under bright-field illumination using a camera lucida.

The injection zone typically consisted of a roughly circular core and a surrounding region in which labeled neuronal processes and perikarya were unevenly distributed. Within a radius of 100 µm, neuronal processes appeared black or dark brown but were too densely packed to permit individual tracings. Between radii of 100 to 2500 µm tracings were made of smooth and beaded axons, spiny and aspiny dendrities, and neuronal perikarya with and without distal dendritic appendages. A marked asymmetry was detected in the distribution of labeled elements. When this pattern was aligned with the best frequency map, the length of neuronal processes along an isofrequency line was significantly greater than along the orthogonal axis. Within a single tissue section, polar analysis revealed little change in the orientation of the labeling pattern. Variations in orientation were seen among different tissue sections from the same brain, although major bins of polar histograms remained oriented parallel to isofrequency lines.

In some experiments, an injection near the middle of an isofrequency line produced dense labeling along only one dimension of constant best frequency. In most cases, at least one major bin of the polar histogram was oriented obliquely to the isofrequency line. These variabilities suggest that orientation of asymmetrical labeling may be related to other physiological properties in addition to tonotopy. (BNS 76-19893, HD-03352, NS-05459, NS-12732)

288.3 RESPONSES TO FM SWEEPS IN BINAURAL FACILITORY (EE) AND BINAURAL INHIBITORY (EI) CELLS IN CAT PRIMARY AUDITORY CORTEX-- J.R. Mendelson, J. McNulty*, and M.S. Cynader, Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada.

The responses to frequency modulated (FM) sweeps were examined in primary auditory cortex of anaesthetized, paralyzed cats. Stimuli were presented (through ear speakers) and responses were collected using a PDP/11 computer. Initially, the characteristic frequency (cf) of each cell was determined using pure tones (i.e. the optimal response to pure tones of a single frequency). Then, FM sweeps, ranging from either low to high frequencies (upward directed) or from high to low frequencies (downward directed), passing through the cf were presented to one or both ears. The speed (i.e. the rate of change in frequency) within each FM sweep remained constant but was varied across conditions.

Tested monaurally, most cells responded well to FM sweeps. Fifty-six % of the units displayed clear direction selectivity with over three times as many units preferring downward directed sweeps over upward directed sweeps. Tested binaurally 56 % of the EE units and 74 % of the EI units were direction selective.

EE and EI cells were tested with binaural FM sweeps, but the relative direction of the FM sweep for the two ears was allowed to vary. In the direction selective EE cells the preferred FM sweep direction was usually the same for each ear tested separately. Either summation or facilitation was observed when both ears were stimulated together with FM sweeps in the same direction while responses were less vigorous when opposite-directed FM sweeps were presented to the two ears. In contrast, the responses of the EI cells were suppressed when like-directed FM sweeps were presented to each ear. For 75 % of the EI cells, this suppression was relaxed for opposite directed FM sweeps to the two ears. This implies a direction selective inhibitory input from the silent ear in EI cells. Approximately half of these cells responded best in the binaural condition when a downward directed FM sweep was presented to the contralateral ear and an upward directed FM sweep was presented to the ipsilateral ear while 25 % preferred the reverse order of presentation and 25 % displayed no such selectivity.

The results show that direction selectivity for EE cells was evident during monaural FM sweep presentations and was similar in the two ears. In contrast, for EI cells direction selectivity in the two ears was not always correlated. Direction selectivity could be observed in the suppressive property of the ipsilateral ear even in the absence of excitatory direction selectivity in the contralateral ear.

288.4 FIELD P OF CAT AUDITORY CORTEX: CODING OF FREQUENCY AND INTENSITY BY SINGLE NEURONS. D.P.Phillips* and S.S.Orman* (SPON: R.M.Benjamin). Dept. Neurophysiology, Univ. Wisconsin, 627 Waisman Center, Madison, WI 53706.

Field P is one of seven physiologically identified fields within the cat's ectosylvian auditory cortex, and is located in the caudal bank of the posterior ectosylvian sulcus (PES). Mapping studies have revealed that P is tonotopically organized with cells of high best frequency (BF) located ventrally and closer to the exposed surface while low BF cells are located dorsally and deep in the sulcus. The properties of single cells in P have not previously been described. In the present study, P was identified by its tonotopic organization in electrode penetrations through the caudal bank of the PES in barbiturate anesthetized cats; the responses of single neurons to monaural and binaural tonal stimuli were studied quantitatively using calibrated sealed stimulating systems.

The majority of P neurons had narrow, V-shaped threshold frequency tuning curves with clearly defined BFs in the range 0.2 to 25.0 kHz. Examination of response rate as a function of the intensity of BF stimuli revealed in over 80% of cases that P cells had nonmonotonic intensity profiles which reached a peak at a best sound pressure level (SPL) and then declined with further increments in intensity. For some neurons, the best SPL was constant across frequency and independent of threshold for the frequencies tested; for other cells, the best SPL was related to threshold at each frequency. Best SPLs ranged from 25 to 80 dB. Sharpness of "tuning" to intensity was quantified by measuring the width of the spike count vs intensity plot at 50% maximum response at BF; these measures were generally in the range of 5 to 40 dB. Minimum response latencies for P cells were in the range of 20 to 45 ms. Recordings from the primary auditory cortex in the same animals confirmed previous descriptions of that field. Only 30% of cells had nonmonotonic intensity functions and minimum latencies were as a rule less than 20 ms.

The frequency tuning of field P cells was comparable to that of cells in fields AI (Phillips and Irvine, J. Neurophysiol., 45: 48-58, 1981) and A (Phillips and Irvine, Brain Res., in press, 1982), but in contrast to both of those fields, P cells had longer latencies and their spike counts were usually nonmonotonic functions of intensity. It is not clear whether this nonmonotonicity is inherent to P or a property of its inputs. The present data, however, suggest a segregation in the auditory cortex of cells according to their coding properties.

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288.5 FIELD P OF CAT AUDITORY CORTEX: BINAURAL INTERACTIONS OF SINGLE NEURONS. S. S. Orman* and D. P. Phillips*. (SPON: C. N. Woolsey). Dept. of Neurophysiology, University of Wisconsin, 627 Waisman Center, Madison WI 53706.

The binaural interactions of single neurons in the posterior field of cat auditory cortex (field P) have not previously been described. In these experiments, the responses of single neurons in field P to both monaural and binaural best frequency tonal stimuli were examined quantitatively in barbiturate-anesthetized cats to which acoustic stimuli were presented using sealed sound stimulating systems.

The majority of field P neurons were binaurally influenced, and commonly displayed summative interactions, i.e., their responses to simultaneous, equally intense stimulation of the two ears were stronger than were their responses to monaural stimulation. Cells of low BF (less than 2.0 kHz) often were sensitive to interaural disparities in the phase of tonal stimuli. This sensitivity was manifested as a cyclical relation of spike count to interaural delay, the period of the cycle in all cases being that of the stimulating waveform. The monaural properties of these neurons were diverse. Some delay sensitive neurons were not excited by independent stimulation of either ear, suggesting that their sensitivity to delay was generated by summative interactions. Other neurons were excited by independent stimulation of one or both ears. For these cells, the relative strengths of responses to dichotic and monaural stimulation varied with interaural delay, so that suppressive interactions dominated at some delays, and summation dominated at others.

The majority of field P neurons displayed spike counts which were nonmonotonic functions of stimulus intensity, particularly for binaural stimuli. In some cases this nonmonotonicity reflected that seen in the monaural spike functions of the same cells. For other neurons, monaural stimuli were ineffective in exciting the cells, or elicited spike count functions which were monotonic functions of stimulus level. For these latter types of cells, the nonmonotonicity appears, therefore, to be a consequence of binaural interactions.

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288.6 ASCENDING PROJECTIONS TO AUDITORY CORTEX IN THE MUSTACHE BAT PTERONOTUS PARNELLII PARNELLII. S.F. Isbey* and J.H. Casseday. Depts. of Surgery (Otolaryngology) and Psychol., Duke Univ., Durham, N.C. 27710.

To continue our investigation of auditory pathways in the bat, Pteronotus p. parnellii, we have examined ascending projections to auditory cortex as seen after injections of horseradish peroxidase (HRP) into subdivisions of auditory cortex. Here we correlate projections with a cytoarchitectural analysis of auditory cortex and thalamus. The auditory cortex occupies most of the lateral surface of the hemisphere and consists of three cytoarchitecturally distinct areas: a "core" of koniocortex and dorsal and ventral belt areas. The medial geniculate body appears to have four divisions. The lateral part of the ventral region (GMvl) is a crescent-shaped area containing small and medium sized cells in a laminar arrangement. The central part of the ventral region (GMvc) contains mainly small cells which are densely packed and arranged in swirls. The medial division (GMm) contains the largest cells in the nucleus. The dorsal division (GMd) is a thin cap containing large elongated cells.

HRP injections in cytoarchitectonically defined areas of auditory cortex result in characteristic patterns of label in GM. GMvc contains large numbers of labeled cells after injections including koniocortex but not after injections of belt areas. GMm however contains the greatest number of labeled cells after injections of the dorsal belt, and also contains a few labeled cells after injection in any part of the auditory field. Preliminary evidence shows that GMvl contains labeled cells after injections in a part of koniocortex that is separate from the target of GMvc. GMd contains at least a few labeled cells after injections in most parts of the auditory field.

The results suggest that (1) GMvc and GMvl project to koniocortex, (2) GMm has a specific target in the dorsal belt but also projects diffusely to the entire auditory field, and (3) GMd projects diffusely to all of auditory cortex. The neocortex of the bat is "primitive" yet in Pteronotus is highly specialized for the bat's auditory behavior. In view of this cortical specialization, the similarity is striking between the bat thalamocortical auditory system and the more general auditory system seen in other mammals such as the cat or tree shrew. Thus the study of functional specializations in the auditory cortex of Pteronotus may be of general significance in understanding the mammalian auditory cortex.

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288.7 FUNCTIONAL ORGANIZATION IN THE ANTERIOR AUDITORY CORTEX OF THE MUSTACHED BAT. D. Wong*, A. Asanuma*, and N. Suga. Department of Biology, Washington University, St. Louis, MO 63130.

The auditory cortex of the Panamanian mustached bat (Pteronotus parnellii rubiginosus) occupies a relatively large part of its cerebral cortex. Previous neurophysiological studies of the auditory cortex in this species have found three major functionally-distinct regions: the DSCF, CF/CF, and FM/FM areas. Each of these physiologically-defined sites is uniquely specialized in processing particular parameters of the biosonar signals and echoes that are utilized by the bat for echolocation.

In a further series of neurophysiological mapping experiments, the response properties of neurons were investigated in a cortical region anterior to the DSCF area and ventral to the CF/CF area. Extracellular recordings of single units or small clusters of units were obtained from unanesthetized bats that were presented with sounds synthesized to simulate their naturally-occurring, emitted orientation sounds and echoes. All of the neurons sampled in the anterior area responded to single pure tones. Most had best frequencies between 90-94 kHz and thresholds ranging from 40-60 dB SPL. These neurons commonly exhibited impulse-count functions that often reached a plateau over an amplitude range as wide as 40 dB. Consequently, there was no basis for amplitude representation in this area. This was in contrast to the DSCF area in which neurons were systematically arranged according to their preferred stimulus amplitudes of maximal response (i.e., best amplitudes). A general tonotopic organization between 90-100 kHz was found in the frequency maps derived from reconstructions of multiple oblique electrode penetrations made in individual animals. The frequency-tuning curves of these neurons were broader than the very sharply tuned DSCF neurons. Moreover, the frequency representation abruptly dropped at the border between the anterior and DSCF areas, since neurons with best frequencies below 90 kHz and above 70 kHz were rarely found. Near the ventral extent of this border a small cluster of combination-sensitive neurons was discovered. Facilitation in neuronal discharge was elicited from stimulation with sound pairs that included the first harmonic of the orientation sound and the second harmonic of an echo. Autoradiographic studies showed that this ventral region received a projection from the CF/CF and FM/FM facilitation areas.

These mapping experiments demonstrate a separate frequency representation in a discrete region anterior to the DSCF area. While the roles of neurons in processsing of biosonar information are known in the CF/CF and FM/FM areas, where frequency representation between 90-94 kHz are also found, the functional significance of the anterior auditory cortex in echolocation remains to be determined. (This research was supported by PHS Grant NS 17333 and Sensory Physiology Training Grant NINCDS 1-T32-NSD 7057.)

288.8 THE AVIAN MOTOR SYSTEM FOR SONG HAS MULTIPLE SITES AND TYPES OF AUDITORY INPUT. L. C. Katz* (SPON: M. Konishi) Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

A songbird cannot develop his species-typical song unless he is able to hear himself vocalize. The brain areas responsible for song production must therefore have access to auditory feedback. The song system consists of a chain of highly discrete nuclei; intracellular recording in one of these, the nucleus hyperstriatum ventrale, pars caudale (HVc), has revealed many neurons that respond to auditory stimuli (Katz, L. C. and Gurney, M. E. Brain Res. 221:192, 1981). I have examined where and in what forms auditory input might be reaching other areas of the song system. Intracellular recordings have been obtained from three areas: 1) the neostriatal shelf area underneath HVc, which contains auditory neurons that send axonal collaterals into HVc; 2) nucleus robustus archistriatalis (RA), which receives a projection from HVc; and 3) the magnocellular nucleus of the anterior neostriatum (MAN).

All experiments were performed on anesthetized adult male zebra finches (Poephilia guttata). Lucifer Yellow filled microelectrodes were used for recording and staining of neurons. Stable cells were presented with noise or tone bursts via a closed sound delivery system. Neurons were filled with dye by passing hyperpolarizing DC current or current pulses.

In experiments on 7 birds, intracellular recordings were obtained from 32 cells in the neostriatal shelf area, of which 17 (53%) showed auditory responses. Of the 17 auditory cells, 11 (65%) had latencies of 100-350 msec; the other 35% had 30-40 msec latencies. Both types of cells had low spontaneous activities (less than 5 spikes/sec). Long latency cells showed a sluggish response pattern in which the duration of the response itself was over 100 msec. All auditory shelf neurons preferred noise bursts to tone bursts of any frequency (1-4 KHz). The morphology of these cells was identical to that described by Katz and Gurney (Brain Res. 221:192, 1981).

In 9 birds, intracellular records were obtained from 24 cells in RA, of which 19 (79%) showed auditory responses. All auditory cells had high spontaneous activity (25-40 spikes/sec) consisting of regularly spaced action potentials. These cells all responded to either noise or tone bursts with a single spike with a 35 msec latency. Unlike auditory neurons in either HVc or the HVc shelf, these cells responded to every stimulus presentation, and responded equally well to noise bursts or tone bursts of 1.7 KHz (the center frequency of zebra finch song). The 4 stained RA neurons recovered were all of the spiny class as described by Gurney (J. Neurosci. 1:658, 1981).

Intracellular recordings were obtained from 16 cells in MAN in 5 birds. In no case was there any sign of auditory responses.

The existence of several different sites and patterns of auditory responses within the song system, coupled with an apparent linkage of auditory responses to distinct morphological and physiological cell types, may reflect the complex, multiple roles of auditory information in the acquisition, development and production of song. (Supported by an NSF Graduate Fellowship.)

288.9 SPECIFICITY AND SELECTIVITY OF NEURONAL RESPONSES TO SONG IN A VOCAL CONTROL NUCLEUS OF WHITE-CROWNED SPARROWS (Zonotrichia leucophrys). D. Margoliash, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Song in oscine passerines is learned, and is sensitive to sensory deprivation both during an acquisition phase, and a production phase. A discrete set of nuclei subserve song production in adult birds. One of these nuclei (HVc - hyperstriatum ventrale, pars caudale) is associated with a "shelf" area that receives a projection from the auditory telencephalon. While HVc and "shelf" neurons have been shown to have auditory responses, the functional significance of these responses remains in doubt. To ascertain the potential relationship between HVc and "shelf" auditory responses and the song learning phenomena, an extracellular single unit study was undertaken.

White-crowned Sparrows were induced to sing by subcutaneous testosterone implants. Songs (typically consisting of four phrases or parts) were recorded, digitized (PDP 11/40), and modeled as frequency and amplitude functions. These functions could be used (with special electronics) to accurately re-synthesize the songs. Frequency information was extracted by means of zero-crossing analysis. Modifications of a function (for example, frequency shift) produced the appropriate change in the song. In this way, a complex stimulus (song) could be systematically modified along all parameter dimensions while recording neuronal responses. For extracellular recording, standard techniques were used with urethane-anesthetized birds. Tone and noise bursts, and the bird's own song, were used as search stimuli.

A number of cells did not respond to tone and noise bursts, but could be driven by song. Often phrase combinations were necessary to elicit responses, the individual phrases or the song played backwards being ineffective. Frequency shifting one or both phrases, and changing the inter-phrase interval duration could abolish the response, as could manipulation of the fine structure (for example, frequency modulation) within the phrases. On occasion, it was possible to effectively model phrase combinations with simple stimuli such as consecutive tone bursts. In such cases units would only respond to the second tone, even when both were of identical frequency, duration, and amplitude. Such units showed a significant degree of selectivity, responding only to the bird's own song and related songs within a large repertoire of White-crown songs. In all cases, the song selectivity of units could be predicted on the basis of their specificity of acoustic parameters. Whether selectivity to the bird's own song is coincidental or is a reflection of some aspect of song learning or maintenance can now be addressed directly at the level of single units by recording from young birds.

I wish to thank M. Konishi, in whose laboratory these experiments were done, for continued guidance. (DM is supported by an NIH training grant and a Pew Memorial Trust grant to MK.)

288.PO AUDITORY CORTEX RESPONSES TO A SEQUENCE OF REVERSED SQUIRREL-MONKEY CALLS. I. Glass* and Z. Wollberg. Dept. of Zoology, Tel-Aviv Univ., Tel-Aviv, Israel, and Dept. of Physiol. and Biophys., Univ. of Washington, Seattle, WA 98195.

The presentation of species-specific calls in a natural sequence has been shown to result in a lower responsiveness of monkey auditory cortex (AC) neurons compared to that elicited by individual calls presented in an isolated manner (Glass, I. and Wollberg, Z., Soc. Neurosci. Abstr. 7: 232, 1981). The present work addresses the question: are these differences in responsiveness the result of a difference in the behavioral significance of the two paradigms? We will show here that the relatively low effectiveness of a sequence of sounds is apparent also when a series of artificial stimuli was presented. Seventy-nine single units were extracellularly recorded from the AC of awake, unmedicated squirrel monkeys. An 82 sec. sequence of 70 natural calls was presented backwards at 75 ± 10 dB spl. This was the reversed version of the natural sequence tested on the same set of cells and reported earlier. The reversed sequence was presented 15 consecutive times, following which detailed analyses were performed on unit responses to 21 representative reversed calls ("llacs"). Eighty-six percent of the cells responded to at least one of the 21 representative llacs with an average of 7.2 effective llacs per neuron. The effectiveness of the reversed sequence in evoking responses was not found to be significantly different from the effectiveness of the natural sequence itself. However, the effectiveness of each of the sequences was significantly lower than that of isolated calls or isolated llacs (the responsiveness to the isolated calls and llacs was measured in another set of AC units--Wollberg, Z. and Glass, I., Neurosci. Letters, Suppl. 7: S70, 1981). The effectiveness in evoking responses of the 21 llacs of the sequence differed significantly from each other. However, no dependency was noted between the percentage of responding units and the average intensity of a llac, its spectral content, or order of presentation. No subgroup of the 21 llacs was found to be distinct in its effectiveness in comparison to the other stimuli. These findings suggest that testing AC neurons with continuous sounds results in a reduced responsiveness compared to their responsiveness to isolated sounds, whether natural or artificial.

289.1 SOMATOTOPIC ORGANIZATION OF THE RAT TRIGEMINAL GANGLION DETERMINED BY HORSERADISH PEROXIDASE (HRP) MAPPING. C. Welt. Waisman Center, University of Wisconsin, Madison, WI 53706.

Somatotopically organized neuronal aggregates, corresponding to the arrangement of vibrissae on the rat's face, are seen at all central levels of the trigeminal somatosensory pathway. The aim of the present study was to determine whether these discretely ordered central and peripheral projection patterns are reflected in the organization of sensory neurons within the ophthalmic-maxillary (OM) division of trigeminal ganglion (TG). The topographic organization, number, and size distribution of first-order TG cells were defined using retrograde axonal transport of HRP. Infraorbital, supraorbital, corneal, and individual vibrissal nerves were transected and HRP crystals applied to the proximal cut end. In other animals, a concentrated HRP solution was injected directly into single vibrissa follicles. Survival times of 4-48 hr were used with 24 hr providing best results. Frozen sections were reacted with either paraphenylenediamine/pyrocatechol or tetramethyl benzidine.

Retrogradely labeled neurons were readily identified for each nerve and follicle studied. Single or small clusters of cells of different sizes were widely scattered among the more numerous unlabeled cells. Although HRP labeled neurons supplying a particular region of the face were generally localized, the extensive distribution of these cells over a relatively large area produced an overlapping somatotopic pattern. Supraorbital and corneal cell bodies were located along the medial side of the ganglion. Primary sensory neurons related to the vibrissae were located in the medial two-thirds of the OM division at all dorsoventral (DV) levels. Cell bodies projecting to individual vibrissal rows were distributed in overlapping diagonal bands through the full anteroposterior (AP) extent of the OM, with row A cells in more medial locations and row E in relatively lateral locations. Distinct aggregates of labeled vibrissa cells were not observed. The lack of discrete segregation of vibrissa neurons was best demonstrated by the fact that some neurons for each row, and even a single vibrissa, were found at most ML, DV and AP levels of the OM division.

The mean diameters and overall size distribution of labeled infraorbital and vibrissa cells were similar to those of the total population of ganglion cells previously measured. These data indicate first, that the precise somatotopic patterns delineated at the central levels of the pathway do not reflect the organization of first-order afferents in the trigeminal ganglion; and second, that the vibrissae are not selectively innervated by a distinct morphological subpopulation.

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289.2 TRIGEMINAL PRIMARY AFFERENT PROJECTIONS IN THE RAT: AN HRP STUDY. M.F. Jacquin, K. Semba, R.W. Rhoades & M.D. Egger. Dept. of Anatomy, UMD-Rutgers Medical School & NJSOM, Piscataway, N.J. 08854

Horseradish peroxidase (HRP) applied to the transected mandibular division of the trigeminal (V) ganglion was transported anterogradely to primary afferent terminal zones in the dorsal brainstem V nuclear complex, and retrogradely to cell bodies in the V motor, supratrigeminal and mesencephalic nuclei. Primary V afferents originating in the V ganglion were also visible in the ipsilateral cerebellar cortex and paraflocculus, and the dentate, cuneate, solitary, supratrigeminal, and dorsal motor vagal nuclei, parvicellular reticular formation, area postrema and C1-C6 dorsal horn, laminae I-V. The contralateral medulla and cervical dorsal horn were also innervated by primary afferents which crossed in the posterior commissure to terminate medially in laminae I,II and V. These projections were also labeled in various combinations when HRP was applied to the lingual, inferior alveolar, mylohyoid or auriculotemporal branches of the mandibular nerve. Transganglionic transport of HRP in the latter 4 cases revealed some intradivisional somatotopy in the ganglion and brainstem. Mylohyoid somata occupied ventroposterolateral portions of the ganglion while auriculotemporal somata were situated dorsoposterolaterally, with lingual somata largely interposed. Inferior alveolar labeling spanned the entire mandibular portion of the ganglion. Terminal labeling was seen in subnucleus principalis, oralis, interpolaris and caudalis in all cases. Intermittent terminal labeling in lingual, mylohyoid and auriculotemporal cases indicated an intradivisional "onion leaf" brainstem topography, especially in caudalis: auriculotemporal fibers terminated most caudally, lingual fibers most rostrally. Inferior alveolar labeling was continuous and uniformly dense throughout all subnuclei. Retrogradely labeled V mesencephalic cell bodies were observed in each of the inferior alveolar, mylohyoid and auriculotemporal cases.

HRP applied to the transected ophthalmic-maxillary division of the V ganglion was transported anterogradely to the remaining portions of the brainstem V complex and retrogradely to cell bodies in the caudal V mesencephalic nucleus. Labeled primary afferents were also visible in the ipsilateral solitary, supratrigeminal and gracilis nuclei, parvicellular reticular formation and C1-C2 dorsal horn, laminae I-V. Lateral laminae I and II of the contralateral cervical dorsal horn were also innervated by primary afferents which crossed in the spinal grey. Mandibular and ophthalmic-maxillary primary afferents overlapped most notably in ventrolateral and central portions of ipsilateral V brainstem subnuclei.

Widespread terminations of the V nerve suggest a functional complexity hitherto undescribed in somatosensory systems (see Jacquin & Zeigler, Br. Res., 238, 1982). Supported by NIH grants NS06419, NS13456, EY03546, EY04710 and NSF grants BNS7824470, BNS8004601.

289.3 MUSCLE AFFERENT INPUTS TO FUNCTIONALLY IDENTIFIED NEURONES IN MEDULLARY DORSAL HORN (TRIGEMINAL SUBNUCLEUS CAUDALIS). N. Amano*, J.W. Hu*, G. Zhong*, and B.J. Sessle. Faculty of Dentistry, University of Toronto, Toronto, Canada M5G 1G6.

Despite the frequent occurrence of muscle pain in the orofacial region, no information is available of the brainstem pathway(s) and organization related to nociceptive transmission from the jaw and tongue musculature. Since subnucleus caudalis is considered an integral component of cutaneous trigeminal nociceptive transmission, it was explored as a possible site receiving muscle afferent information in nine chloralose-anaesthetized cats. Extracellular recordings were made from single neurones which were first examined in terms of their electrically evoked inputs, and then functionally identified on the basis of their cutaneous afferent inputs as being low-threshold mechanoreceptive (LTM), wide dynamic range (WDR), or nociceptive-specific (NS). In addition to the use of electrical and natural (tactile, pinch, heat) cutaneous stimuli, electrical stimulation was applied to the ipsilateral hypoglossal and temporalis nerves to excite tongue and jaw muscle afferents. Small branches of the lingual and temporalis arteries were also cannulated to test for possible effects on the neurones of the intra-arterial injection (0.4-0.6 ml) of 7% NaCl, KCl (80 mM), bradykinin (20 μg) and histamine (40 μg); 0.9% NaCl was injected as a control solution.

A total of 42 LTM, 13 WDR and 25 NS neurones was studied. Their cutaneous receptive field properties and loci were comparable to those previously reported in cat (Hu et al., J. Neurophysiol. 45, 173, 1981). Electrical stimulation of muscle afferents activated only 7 of 42 LTM neurones tested. In contrast, 10 of 13 WDR neurones and 19 of 25 NS neurones were excited, at latencies ranging from 6-25 msec. Their latencies and high threshold for electrically induced activation were suggestive of muscle afferent input predominantly from high-threshold muscle afferents. This is further suggested by our findings that 15 of 22 nociceptive neurones tested could be excited by the injection of the noxious chemicals: 8 neurones were excited by three or four of these chemicals, and 7 by two. No neurones have been encountered to date that could only be excited by stimulation of muscle afferents. These findings indicate that many of the cutaneous nociceptive neurones within subnucleus caudalis are also concerned in the relay of muscle nociceptive information from the jaw and tongue musculature.

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289.4 ORGANIZATION OF PROJECTION NEURONS IN THE MEDULLARY DORSAL HORN. W. M. Panneton and H. Burton. Dept. Anat., St. Louis Univ., St. Louis, MO 63104 and Dept. Anat. and Neurobiol., Washington Univ., St. Louis, MO 63110.

Neurons located within the subnucleus caudalis (the dorsal horn of the medulla) and the subnucleus interpolaris project to multiple targets including the thalamus and rostral trigeminal areas. Our recent studies have begun to dissect the organization of these trigeminal projection neurons using the retrograde transport of either horseradish peroxidase or fluorescent dyes.

Anesthetized cats were prepared for stereotaxic surgery; electrophysiological recording techniques often were used to confirm stereotaxic coordinates before injections. In initial experiments, 1% WGA-HRP was injected by pressure via micropipettes into rostral trigeminal areas. Injections centered in caudal and ventral parts of the principal trigeminal nucleus but including rostral parts of the subnucleus oralis resulted in numerous retrogradely-filled neurons ipsilaterally in laminae III and IV of the medullary dorsal horn; some also were present in laminae I and V, bilaterally, and in lamina II, ipsilaterally.

In contrast, a more rostral injection including the medial parabrachial nucleus and the dorsal principal nucleus resulted in numerous labeled neurons in laminae I and V but few labeled neurons in laminae III and IV ipsilaterally. Some reactive neurons were located in outer parts of lamina II, ipsilaterally, and in laminae I and V, contralaterally.

Since neurons in lamina I also have a dense projection to nucleus submedius (Craig and Burton, '81), double-label experiments were done to test if the same neurons project to both medial thalamus and rostral brainstem targets. Multiple injections of the fluorescent dye fast blue (FB) were placed in the medial thalamus. After 13-25 days nuclear yellow (NY) was injected into the parabrachial-rostral principal trigeminal area on the opposite side. Numerous neurons in lamina I were labeled with either FB or NY. Those labeled with FB were located preferentially in the pockets of lamina I neuropil interstitial to the spinal trigeminal tract, especially laterally, while those labeled with NY were mostly along the medial edge of the spinal trigeminal tract, especially in dorsomedial areas. Few double-labeled neurons were seen. (Supported by grants PHS NS09809 and PHS 5 S07 RR05388-20.)

289.5 EFFECTS OF KNIFE-CUT LESIONS OF ROSTRAL AND CAUDAL TRIGEMINAL PROJECTIONS ON NOXIOUS AND NON-NOXIOUS OROFACIAL STIMULATION. J.G. Broton and J.P. Rosenfeld. Dept. of Psychology, Northwestern University, Evanston, Ill. 60201.

The trigeminal sensory nuclear complex (V-NUC) is an elongated nuclear mass in the lateral medulla, from which emanate projections conveying somatosensory information from the face. Primarily due to clinical observations, it is widely believed that rostral projections convey touch and "epicritic" sensation while pain and "protopathic" sensation are transmitted by projections leaving caudally. Recently, however, we have shown that rostral projections are necessary for normal facial nociception in the rat, suggesting a different functional organization of V-NUC-efferent projections. To further elucidate this organizational pattern, the present study tested the effects of interruption of rostral or caudal projections on responses to both noxious and non-noxious stimuli applied to five different areas of the face.

Adult male Holtzman albino rats were used. The test of non-noxious responsivity involved monitoring the awake rat's cortical arousal level via a surface-to-depth bipolar electrode implanted in somatosensory cortex. During testing the rats were tail-restrained and kept in a shielded box. The cortical record was observed until 10 to 20 second synchronization bursts occurred. Stimuli were presented to the face using devices connected to a socket attached to the rat's skull. Low-level 5-second air puffs were presented during synchronization, and air pressure was adjusted for each of the five facial sites to a level which reliably produced cortical desynchronization. Heat applied to each site was used as the noxious stimulus. Heat was adjusted to produce a nocifensive face-rub response (FRR) of reliable latency. After several days of baseline testing, at which time air pressure and heat levels were set, sagittal cuts were made medial to V-NUC by lowering a curved piece of tungsten wire through a chronically implanted guide cannula. The wire was directed either rostrally or caudally, cutting different rostrocaudal levels of V-NUC-efferent projections. Rats were retested 24 hours after the cuts.

Preliminary results indicate that, where FRR latencies were significantly elevated after rostral cuts (medial to main sensory nucleus and nucleus oralis), detection of air puff stimuli occurred at the pre-cut intensity setting. Work in progress will assess caudal cut effects, as well as somatotopic patterns of effects across the five sites tested. (Supported by NIH Grants GM23696 and DE05204 to J.P.R.)

289.6 RESPONSES OF NUCLEUS GRACILIS NEURONS TO ACTIVATION OF GUARD HAIR RECEPTORS BY STIMULI MOVING ACROSS THE SKIN. A. J. Castiglioni and L. Kruger. Depts. of Anatomy and Anesthesiology, Ahmanson Lab. of Neurobiology, and the Brain Research Institute, Center for Health Sciences, UCLA, Los Angeles, CA 90024.

Recordings of single neuronal activity in the gracile nucleus of barbiturate-anesthetized rats were obtained with tungsten wire microelectrodes. The limits of receptive fields (RF) were determined with fine tipped probes and the neuron's responses to multiple presentations of a microprocessor-controlled fine air jet moving linearly across the field at constant velocity were recorded. The air jet was moved along the same path over the RF at several velocities and in both directions for each orientation. The units studied responded to bending of single guard hairs or tufts of hairs with a response of one or a few action potentials. Most units fired occasional 'spontaneous' action potentials at irregular intervals (less than one/sec). The RFs were located on the dorsal hindlimb, dorsal abdomen, and dorsal-caudal thorax. Each traverse of the moving airjet produced a series of action potentials with a characteristic temporal pattern. Responses varied little between trials across a given path over the RF. However, analysis of responses to stimulus traverses at different velocities revealed that the neurons fired action potentials when the airjet had reached specific positions in the RF. Thus, the temporal pattern of firing may be a manifestation of the distribution of those hairs within the RF with connectivity to the cell being recorded and the gradients of sensitivity within the RF. The response to air jets moving along the same path often differed with the direction of movement, but, in many cases, the temporal pattern of spiking was similar in opposite directions, although reversed. However, the number of spikes elicited in each direction often displayed consistent differences and the temporal pattern reversal was difficult to assign to identical spatial coordinates. The length and orientation of guard hairs appears to be a factor in the apparent directional difference. Supported by NIH Grant NS-5685.

289.7 CONTRASTING PROPERTIES OF RACCOON CUNEOTHALAMIC AND CUNEOCEREBELLAR RELAY NEURONS. Benjamin H. Pubols Jr., John H. Haring*, and Mark J. Rowinski. Department of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA 17033.

The main cuneate nucleus (MCN) of the raccoon, like that of the cat (Kuypers & Tuerk, 1964; Keller & Hand, 1970), consists of cytoarchitecturally distinct cell clusters and polymorphic regions. In the present study, extracellular unit activity of 163 raccoon MCN units was recorded in response to natural stimulation of their peripheral receptive fields (RFs), and to electrical stimulation of the contralateral thalamic ventrobasal complex (VB), and either the ipsilateral restiform body or cerebellar anterior lobe (Cblm).

A total of 68 units were thalamically activated (TA units), 51 antidromically (cuneothalamic relay, or CTR, cells), and 17 transynaptically; 5 of the CTR units were also transynaptically activated. A total of 98 units were cerebellar activated (CA units), 31 antidromically (cuneocerebellar relay, or CCR, cells), and 67 transynaptically. Of 45 units tested, 3 could be activated by both VB and Cblm stimulation; however, none was antidromically activated from both sites. CTR neurons were located throughout the entire rostrocaudal extent of the clusters region, while CCR cells were located in polymorphic regions, primarily in the ventral polymorphic region in the first 2 mm rostral to the obex (the "medial tongue" of Johnson, Welker, & Pubols, 1968).

TA and CA cells differed functionally in the following ways: (1) Nearly all TA cells had cutaneous RFs, while only about 35% of CA cells did, the remainder being activated by stimulation of deep receptors, in particular by muscle stretch; (2) All TA cutaneous units had RFs on glabrous skin, while 60% of CA cutaneous RFs were on hairy skin; (3) Glabrous skin RF areas of CA units tended to be smaller than those of TA units; (4) A bursting discharge pattern (either spontaneous or evoked) was characteristic of TA units, while a pattern of irregularly spaced single spikes was characteristic of CA units.

In contrast to the exquisite representation of the glabrous skin of the raccoon's hand in the CTR system, light touch appears to form only a minor component of the CCR arising from neurons of the MCN. The modality composition of MCN neurons projecting to the cerebellum is similar to that of the cat external cuneate nucleus (Cooke, Larson, Oscarsson, & Sjölund, 1971). Thus, it is suggested that, in the raccoon, the medial tongue of the MCN functions primarily as a subnucleus of the external cuneate nucleus, relaying to the cerebellum information arising mainly from deep receptors, including proprioceptive information originating in muscles of receptors concerned with hand movements. (Support: NS-13418, USPHS.)

289.8 INTERACTIONS OF SEPARATED VENTROLATERAL COLUMN (VLC) AND DORSAL FUNICULAR (DF) INPUTS INTO CUNEATE NEURONS IN DECEREBRATE DECEREBELLATE CATS. S. J. Jabbur, N.E. Saadé*§ and N. R. Banna*§. Dept. of Physiol., Fac. of Med., Amer. Univ. of Beirut and §Fac. of Sci., Lebanese Univ., Hadath-Beirut, Lebanon.

Fifty cuneate neurons were recorded in anesthetized decerebrate and decerebellate cats with one of two types of spinal lesions effected at both C_1 and C_3 levels. One type of lesion involved bilateral transections of the dorsal funiculi and dorsolateral fasciculi (DF-DLf cuts), thus leaving the ventral tracts intact. The other type of lesion consisted of transections of the ventral and lateral funiculi on one side (VF-LF cuts) coupled with DF-DLf transections on the other side. In addition, the first three dorsal roots were transected bilaterally in all cats. Searching test stimuli consisted of either ipsilateral peripheral shocks on the side of the VF-LF cuts, or ipsilateral DF shocks rostral to DF-DLf cuts. Conditioning electrical stimuli applied to the contralateral VLC caudal to the DF-DLf cuts inhibited about half the cuneate neurons responding to the test ipsilateral stimuli. Inhibition was apparent in cuneothalamic neurons and interneurons. This inhibitory VLC input could be mediated via the brain stem reticular formation or raphe nuclei, which have been shown to inhibit the cuneate nucleus (Cesa-Bianchi, M.G. and Sotgiu, M.L., Brain Res., 13:129, 1969; Dostrovsky, J.O., Brain Res., 200:184, 1980). Our findings provide further evidence against the strict segregation of sensory activity traveling along the somatic sensory pathways (Supported by grants from the Lebanese National Research Council).

289.9 PROJECTIONS TO THALAMUS FROM EXTERNAL CUNEATE NUCLEI AND CELL GROUPS X AND Z,AS WELL AS FROM CUNEATE AND GRACILE NUCLEI, IN RACCOONS. E.-M. Ostapoff* and J. I. Johnson. Depts. of Psychol. and Anatomy and Neuroscience Progr., Michigan State Univ., East Lansing, MI 48824.

To determine the full extent of possible medullary inputs to the postcranial body projection region of the somatic sensory thalamus, large injections (1 - 2 µl) of 30% horseradish peroxidase (HRP) were injected into the thalamus of 7 raccoons immobilized with ketamine and anesthetized with Dial-urethane. As determined by preliminary explorations with recording tungsten microelectrodes, the injections were centered in the somatic sensory receiving region of the ventrobasal complex, pars externa, responsive to stimulation of the postcranial body. Following 3 to 4 days survival the brains were processed to show HRP using tetramethylbenzidine and diaminobenzidine as chromogens in alternate series of 40 µm sections in horizontal or transverse planes. In the thalamus, injections appeared to cover most or all of the ventrobasal pars externa and some surrounding tissue, but were confined to one hemisphere. In the medulla, in addition to the well known massive projections from the contralateral cuneate and gracile nuclei, retrogradely labelled cells were found in a group of small cells dorsal to the caudal pole of the contralateral descending vestibular nucleus, corresponding in cell size and location to cell groups x and z described in the cat (Brodal, A. & Pompeiano, O., J. Anatomy 91:438, 1957). Also labelled were cells in the medial one third of the contralateral external cuneate nucleus. A similar thalamic projection from the external cuneate has been reported in monkeys (Boivie, J., et al. Neurosci. Letters 1:3, 1975) but was not seen in cats (Cheek, M. D. et al., J. comp. Neurol. 164:31, 1975). In raccoons we also found substantial numbers of labelled cells in the contralateral lateral reticular nucleus pars lateralis, and in the contralateral lateral cervical nucleus. Subsequently, in 2 raccoons, very small injections, applied from the tip of a recording tungsten microelectrode (Johnson, J. I. & Hill, J. M., Anat. Rec. 196:236, 1980), were made while recording from units responding to mechanical displacement of muscle masses in the lower arm, in the kinesthetic region at the dorsorostral pole of the sensory thalamic region (Wiener, S. I. et al., this vol., 1982). Following the same survival times and tissue processing, labelled cells were again seen in the medial part of the external cuneate, and in ventral portions of the cuneate nucleus, contralateral to the injection.

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289.10 ACETYLCHOLINESTERASE (AChE)-DEPENDENT STAINING IN INFANT RAT VENTROBASAL THALAMUS. J. Solomon* and D. Kristt (SPON: B. Ransom). Dept. Pathology, Div. Neuropathology, Stanford Univ. Med. Ctr., Stanford, CA 94305.

The ventrobasal complex (VBC) of the thalamus contains neurons providing the last link in the somatosensory pathway to neocortex. These cells are believed to give rise to axons which terminate in barrel-like clusters of neurons in layer IV of primary somatosensory cortex (SmI). Previous work on the development of staining in rat brain [JCN 186 (1979) 1; Anat.& Embryol. 163 (1981) 31] suggested that these VBC neurons give rise to AChE-rich axons terminating in SmI cortex. However, histochemical studies on adult rat thalamus, showed this region to be poorly stained for AChE (e.g. Jacobowitz & Palkovits '74). In undertaking the present study it was postulated that there was a developmental loss in AChE staining of this region of thalamus. This hypothesis has been verified. However, the two components of the nucleus, viz. arcuate and external portions, have different developmental time courses for these histochemical changes. The method used for AChE histochemistry has been described (JCN 186:1). Alternate Nissl sections helped in evaluating the nuclear organization of thalamus.

From birth to 6 days of age (6dpn) both the arcuate and external components of the VBC are densely stained. Staining of both neuronal somata and neuropil is seen. Surrounding thalamic nuclei are unstained. By 16 dpn, the arcuate portion of the VBC is somewhat more lightly stained for AChE than the external portion. The VBC at 19 dpn shows almost complete loss of staining in the arcuate portion of the nucleus; slight staining is present ventrolaterally in the external portion. By adulthood, virtually the entire VBC is unstained, in agreement with previous observations.

Hence there are differences and similarities in the maturational changes in AChE staining of VBC and SmI. They are similar in that loss of staining occurs towards the end of the third week postnatally. The major difference is the progressive loss in staining in the VBC, particularly the arcuate portion that begins much earlier than any apparent alteration in layer IV of SmI. At the latter site the loss of AChE positivity occurs abruptly within 2-3 days.

There are two principal implications of these findings. First, the relatively high levels of histochemically demonstrable AChE in the VBC at 6 dpn, compared to adjacent nuclei, would further support a previous suggestion that the AChE-positive plexus in the barrel centers of the infant rat and mouse derive from cell bodies in the VBC. Because of the transient nature of AChE staining in this thalamocortical pathway, we speculate that the AChE may exert a trophic effect on neuronal development in somatosensory cortex.

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289.11 SOMATOTOPIC ARRANGEMENT OF SENSORY PROJECTIONS IN THE KINESTHETIC THALAMUS OF RACCOONS. S. I. Wiener*, J. I. Johnson, and E.-M. Ostapoff*, Depts. of Biophysics, Anatomy and Psychology and Neurosci. Progr., Michigan State Univ., East Lansing, MI 48824.

Raccoons have a loose skin (allowing the deposition of a large amount of subcutaneous fat in autumn) over most of the trunk and limbs (but not on the hands, hind feet, tail and face). In these loose-skinned regions it is possible to determine whether mechanoreceptive fields lie within the skin, or are in deeper tissues (muscles, tendons, joints), by moving the skin away from the deeper tissues and determining if the field moves with the skin or remains with the deeper tissues. We used this property to determine that at the rostrodorsal cap of the somatic sensory region of the raccoon thalamus, there is a zone responsive to mechanical displacement of deep tissues of the trunk and limbs. No responses to stimulation of skin or hair were found in this zone. In 8 raccoons, immobilized with ketamine and anesthetized with Dial-urethane, we used tungsten microelectrodes to record evoked activity (unit clusters) in a fine grid (0.1-2.0 mm spacings) of microelectrode penetrations. The dorso-rostro-lateralmost part of the somatic sensory thalamus contained projections from deep tissues of the trunk; the caudal trunk was represented lateral to the rostral trunk. Ventral and caudal from this, laterally, there were projections from the deep tissues of the upper (proximal), then the lower (distal) hindlimb. Medial to these hindlimb projections, a field of responses to deep forelimb stimulation formed a dorsoventrally flattened band which overlay the large representations of cutaneous, claw and hairy portions of the forepaw digits. Within this deep forelimb zone, responses to more distal parts of the forelimb were found ventral and caudal to those of more proximal parts. This kinesthetic region of sensory thalamus is in agreement with studies showing similar regions in squirrel monkeys (Dykes, R. W. et al., Neurosci. 6: 1687, 1981), macaques (Maendly, R. et al., J. Neurophysiol. 46: 901, 1981) and humans (Ohye, C. & Narabayashi, H., J. Neurosurg. 50: 290, 1979).

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289.12 RESPONSE OF SOMATIC NEURONS IN THE SUPERIOR COLLICULUS TO MOVING TACTILE STIMULI. H. Ruth Clemo and Barry E. Stein. Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Visual cells of the superior colliculus (SC) have been shown to be best activated by moving stimuli and to code the direction and velocity of movement (see Stein, B.E. and Gordon, B., Dev. Percept., 2:157, 1981). Presumably, these properties are important in the role this structure plays in visual orientation and localization. While tactile cells in the SC are thought to play a role in orientation and localization, the receptive field properties of these cells are poorly understood. In the present study we sought to determine if tactile cells could also code velocity and direction of movement.

Neuronal recordings (n=85) were made with glass-coated tungsten microelectrodes in 10 cats which were paralyzed and artificially respired with N_2O (75%) and O_2(25%). When an isolated cell was located, the minimal stimulus for activation was identified and the receptive field was mapped with that stimulus. The vast majority (71%) of the cells encountered were activated by gentle cutaneous stimuli (e.g., air streams). Cells with cutaneous receptive fields were then tested by moving a mechanically driven camel's hair brush across the receptive field in opposing directions of a given axis at a pre-determined velocity. After 10 trials, the stimulus velocity was changed and the tests were repeated. After several velocities were presented, the axis of movement was changed and the entire series of tests was repeated. An attempt was made in each cell to move the stimulus along 4 axes separated by 45°. Quantitative evaluation of the effectiveness of these different stimulus parameters was accomplished by comparing the mean impulse frequencies in each of the different conditions. Twenty-six cells were tested with at least 2 velocities applied along at least 3 axes (6 directions). In the majority of these (n=20), rapidly moving stimuli (20 cm/s) evoked higher impulse frequencies more often than either intermediate (10 cm/s; n=6) or low (4 cm/s; n=0) velocities regardless of direction of movement. In addition, most cells showed preferences for a given axis or direction of movement and the magnitude of the preference depended upon stimulus velocity. Curiously, of 20 cells studied with receptive fields on the dorsal aspect of the limbs or paws, 55% preferred movements along the horizontal axis (which is approximately at right angles to the direction of the hair growth). This is also along the same axis of movement most frequently preferred by visual SC cells.

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289.13 COMPLEX SOMATOSENSORY RECEPTIVE FIELDS IN THE DEEP LAYERS OF THE HAMSTER'S SUPERIOR COLLICULUS. R. W. Rhoades[1,2], M. Jacquin[2] and R. D. Mooney[1]. New Jersey School of Osteopathic Medicine[1] and Rutgers Medical School[2], Piscataway, NJ 08854.

Recordings from the deep laminae of the mammalian superior colliculus in a variety of species (eg. Stein, B.E. et al. J. Neurophysiol., 39:401, 1976; Dräger, U.C. and Hubel, D.H., J. Neurophysiol., 39:91, 1976) have revealed large numbers of cells which discharge phasically in response to low threshold (LT) tactile stimuli. More recently, it has been shown (Stein, B.E. and Dixon, J.P., Brain Res., 158:65, 1978) that some deep layer neurons also respond to noxious (NX) somatosensory stimulation. We have used single unit recording techniques to assess the responses of 127 deep layer somatosensory cells in pentobarbital and urethane anesthetized hamsters and have encountered a number of units with receptive field types that have not heretofore been reported for the colliculus.

Of the 127 cells tested, 42% (N=53) yielded simple phasic responses to innocuous stimuli, 12% (N=15) responded only to noxious (NX) stimulation, and 10% (N=13) were classified as having a wide dynamic range. Units with more complex receptive field (RF) types included those in which LT (3%, N=4) or NX (5%, N=6) stimulation only suppressed spontaneous discharges, and neurons which exhibited clearly antagonistic interactions between mechanical stimuli delivered to different points on the body surface. For most of the latter cells (17%, N=21) the interaction was between LT and NX stimulation. A given unit usually had a small excitatory RF in which LT stimulation would increase the resting discharge and a larger RF where NX stimuli would suppress spontaneous activity. A smaller number of cells (9%, N=12) exhibited such interactions between innocuous stimuli, and a very few (2%, N=3) had opposing responses to NX stimulation of different parts of the body. Units recorded in the stratum griseum intermediale (SGI), in most cases (86%), had simple RF's, and this was also true for 75% of the cells recorded in the stratum album intermedium (SAI). Forty percent of the units recorded in the stratum griseum profundum (SGP) had complex receptive fields. The fact that the percentage of units with complex somatosensory RF's was much greater in the SGP than in the SGI suggests that different aspects of the tactile input to the colliculus may be processed in these two laminae.

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289.14 ELECTRORECEPTION IN CHIMAERA (HOLOCEPHALI): RESPONSES IN THE CNS. Theodore H. Bullock and David A. Bodznick. Neurobiology Unit, Scripps Inst. Oceanog., Dept. Neurosci., Sch. Med., U.C.S.D., La Jolla, CA 92093 and Dept. Zool., Wesleyan Univ., Middletown, CT 06457 and Friday Harbor Lab., Univ. Wash.

Fields and Lange (SCI., 207:547-8, 1980) found that chimaeras (Hydrolagus colliei) have electroreception by conditioned behavior to electric fields <0.2 μV/cm, and by recording from units in the lateral line nerve sensitive to feeble currents. We have recorded evoked potentials (EPs), multiunit activity (MUA) and single unit spikes responsive to such stimuli in the medulla, midbrain and cerebellum. Under MS222 and curare ratfish were fixated, the spinal cord transected and cerebellum exposed. Stainless steel electrodes allowed deposition of iron in significant loci.

Clear EPs and unit spikes are obtained with homogeneous field pulses of 5-10 ms and 5-10 μV/cm in an optimum axis and polarity specific for each locus; threshold (averaging 512 trials) is <0.1 μV/cm; 64 μV/cm is near saturation. In the medullary center the EP is a sequence of waves beginning with a large positive peak at ca. 32ms (P32), followed by N38, P55, a large N65, P70, N100, and a large P140; EP amplitude reached 100 μV, (cf. light flash 700 μV). In the midbrain below a reversal depth at 1 mm the sequence in a good example is N40, P80, N125, N160, P250. The first cerebellar deflection is later: N60 or P80 in two sites. MUA bursts occur on peaks out to 225 ms. Some single units show excitation at 30-50 ms in the best polarity; inhibition with rebound discharge in the other; adaptation takes seconds. One unit followed down to 1 μV/cm, saturated at 10 μV/cm, and had a best frequency at 2 Hz. Pulses longer than 25 ms give ON and OFF responses; pulses shorter than 5 ms require substantially higher intensity. In medulla and midbrain repetition rate of brief stimuli has no great effect until >10 Hz; higher rates depress. Trains of 7 stimuli separated by 2s of rest reveal characteristic successions of facilitation and depression. Sine wave stimuli give complex EP forms maximal at 10-20 Hz, varying with the locus; sometimes a frequency doubling at 10 Hz is seen, which reduces the amplitude. EPs show, better than units or behavioral threshold, that frequency is not a scalar which can be compensated for by intensity; wave forms are distinctive for each frequency. Different sites have somewhat different receptive fields, defined by responsiveness of ampullary pore groups.

Flash stimuli elicit sharp peaks in the tectum much like those in other fishes but with long latency; the first peak is P140 (latency ca.= lamprey; ca. 2X shark, 4X catfish), followed by N180 and sometimes N330, N530 and N750; only some of these reverse with depth. (Aided by NIH and NSF grants to T.H.B. and NIH Fellowship to D.A.B.)

289.15 DIENCEPHALIC RESPONSES TO ELECTRORECEPTIVE INPUT IN THE THORNBACK RAY, PLATYRHINOIDIS TRISERIATA. Jeff Schweitzer. Neurobiol. Unit, Scripps Inst. of Oceanog., U.C.S.D., La Jolla, CA 92093

Behavioral and electrophysiological experiments have demonstrated that elasmobranchs respond to weak electrical voltage gradients as low as 0.005μV/cm. Electrophysiological studies have concentrated on the dorsal nucleus, where electroreceptive input first reaches the central nervous system. Preliminary evidence has shown responses to weak electric fields up to the forebrain, but the localization and characterization of electroreceptive nuclei in higher brain centers has not been well established.

In the present study, evoked potentials (EPs) and multiunit activity (MUA) were recorded from the midbrain and diencephalon of the thornback ray, Platyrhinoidis triseriata, in response to electrical pulses delivered to the water bath as a quasi-homogeneous electric field.

The diencephalic EP is maximal between 1.9-2.2 mm below the surface in the caudal ventrolateral diencephalon and comprises a characteristic monophasic wave with a latency of 38 msecs plus labile later peaks. Multiunit activity, phase-locked to the stimulus, often appears at the depth of maximal EP but the depth relationship between EPs and MUA is not necessarily simple.

In the torus semicircularis, the largest response was recorded 0.65-0.70 mm below the surface in a region which may correspond to the toral subdivision called the lateral nucleus of the lateral mesencephalic nuclear complex described anatomically in Raja eglanteria (Boord and Northcutt 1982. J. Comp. Neurol. In press) as verified by electrolytic lesions. The toral EP is also mainly an initial monophasic peak at a latency of 25 msecs. In both regions, maximal responses were recorded at 40μV/cm, 10 msec duration, 1 per 4 sec repetition rate, with a field orientation between 300-330° (0° is rostral midline; 90° is to the left).

It is clear that the response recorded in the diencephalon is generated locally and is not electrotonic spread from the torus. The latency of the first peak of the diencephalic response is 10-15 msecs greater than that in the torus. In the diencephalon, moving the recording electrode vertically less than 1 mm halves the EP amplitude and changes the form of the response. Lesioning the region of maximal response in the diencephalon greatly alters the shape of its EP and reduces the response amplitude. In addition, no MUA was recorded after lesioning the region of maximal activity.

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289.PO MORPHOLOGICAL EVIDENCE OF FIBER SORTING IN THE DORSAL COLUMNS: A COMPARATIVE STUDY. B. C. Albright, J.L. Culberson and L.M. McCann*. Depts. of Anatomy, University of North Dakota, Grand Forks, ND 58201 and West Virginia University, Morgantown, WV 26505

Individual cervical rhizotomies were made in the lesser bushbaby, racoon, tree squirrel, brush-tailed possum and potoroo. Animals were killed after 5 to 7 days survival and cervical and medullary sections were stained for fiber degeneration. In all species, there was morphological evidence of fiber sorting in the cuneate fasciculus (CF) and a related differential termination of primary fibers in the cuneate nucleus (CN). Each lesion resulted in the formation of a single lamina of degenerated ascending fibers in the cervical cuneate fasciculus. In the medulla, a smaller fiber bundle or concentration of degenerated fibers was present medial to the initial lamina following individual lesions of most of the brachial cord levels. The medial bundle appeared to be formed by the medial segregation of some fibers from the dorsal aspect of the original or lateral lamina. In the majority of cases, the two laminae were distinctly separated by normal fibers throughout most of their course in the medulla. Unlike the others, in the bushbaby and squirrel, the formation of the medial bundle occurred within the cervical levels. In all cases, the medial bundle was smaller than the first and, although it varied in shape, it was always superficial in location. Collectively, the medial bundle exhibited significant segmented overlap and appeared in some species as a shallow band of debris which extended transversely along the entire superficial surface of the fasciculus. Caudorostrally, the medial bundle remained relatively unchanged in position and at rostral levels was present dorsal to the cuneate nucleus. The lateral lamina became displaced more laterally at rostral levels and for C_4 to C_7 segments was present ventrolateral to the LCN. Both bundles appeared to contribute varying amounts of preterminal debris to the CN. Fibers in the lateral lamina projected to well defined terminal zones on the CN in some species and in the LCN in all. The organization of these zones reflected the expected segmentotopic organization of the DCN primary input. The medial bundle projected in a more diffuse overlapping manner to the dorsal CN areas. Presently there is no clear functional explanation for the particular type of fiber sorting observed for the cervical primaries in these animals. The pattern of termination tends to be more consistent with reports on the segregation of superficial versus deep modalities in the CN. It seems less likely to represent some form of somatotopic reorganization although the two are closely related.

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290.1 DELTA-9-TETRAHYDROCANNABINOL : BIPHASIC INTERACTION WITH BOVINE SERUM ALBUMIN. S.J.Haque*, S.K. Ray*, J.J. Ghosh* AND M.K. Poddar. Department of Biochemistry, University of Calcutta, Calcutta-700019, INDIA.

The fluorescence probe 1-anilino-8-naphthalene sulphonic acid (ANS) was taken as a tool to study the interaction of delta-9-tetrahydrocannabinol (THC) with bovine serum albumin (BSA). The fluorescence intensity of BSA-ANS complex was significantly reduced in the presence of low concentration of delta-9-THC (1.66 -6.66 μM) but at higher concentration of delta-9-THC (>6.66 μM) no such measurable increase in the quenching of fluorescence intensity of BSA-ANS complex was observed. The emission maxima of BSA-ANS fluorescence were not shifted in the presence of even higher concentration (33.33 μM) of delta-9-THC. Kinetic study reveals that at low concentration of delta-9-THC Km of ANS to BSA was significantly increased without any change in the limiting fluorescence at infinite ANS concentration whereas at higher concentration of delta -9-THC both Km and limiting fluorescence at infinite ANS concentration were altered. The quenching of fluorescence intensity of BSA-ANS complex at low concentration of delta-9-THC was decreased when BSA concentration was increased. Number of moles of ANS bound per mole of BSA (n) was found to be 5.0 in the absence of delta-9-THC and this n value was gradually reduced with the increase of delta-9-THC concentration (0 - 6.66 μM).

These results suggest that delta-9-THC interacts with BSA in a biphasic manner (a) at low concentration it interacts with BSA hydrophobically and inhibits the binding of ANS to BSA competitively, (b) at higher concentration (>6.66 μM) other type of binding is expected. (Supported by the grant from ICMR, New Delhi, INDIA).

290.2 CHRONIC PHENCYCLIDINE DECREASES STRIATAL [^{3}H]-SPIPERONE BINDING AND PRODUCES TOLERANCE TO ITS ACUTE PROLACTIN-SUPPRESSIVE EFFECT IN RATS. D. Lozovsky*, C. F. Saller*, C. C. Chiueh, M. A. Bayorh* and I. J. Kopin. Lab. of Clin. Sci., National Institute of Mental Health, Bethesda, MD. 20205.

Chronic treatment with phencyclidine (PCP) has been reported to decrease (Robertson, Eur J Pharmacol 78: 363, 1982) or to have no effect (Leelavathi et al., Commun Psychopharmacol 4: 417, 1980) on the number of striatal dopamine (DA) receptors in rats, as measured by [^{3}H]-spiperone binding. In the present study, 24 hrs. after 28 days of administration of PCP (10 mg/kg daily, s.c.) to male Sprague-Dawley rats, there was an 18% decrease in the B_{max} for [^{3}H]-spiperone binding to striatal membranes as compared to saline-treated rats (21.4 + 1.1 pmole/g tissue, n=7, and 26.2 + 0.9 pmole/g tissue, n=4, respectively, P<0.01). No significant change in affinity (K_d) was found after chronic treatment with PCP. PCP (1nM-100 μM) added to striatal membranes did not alter [^{3}H]-spiperone binding.

We have shown recently that acute s.c. administration of PCP caused a dose-dependent suppression of the plasma prolactin (PRL) levels in rats (Saller et al., Eur J Pharmacol, submitted). In the present study, plasma PRL in naive rats sacrificed 30 min. after PCP administration (10 mg/kg, s.c.) was decreased by 60% as compared to saline-treated rats (1.24 + 0.37 ng/ml, n=9, and 3.08 + 0.6 ng/ml, n=7, respectively, P<0.05). There was, however, no change in plasmma PRL in rats treated chronically with PCP for 28 days and sacrificed 24 hrs. later, as compared to saline-treated controls (2.76 + 0.76 ng/ml, n=18, and 2.97 + 0.67 ng/ml, n=31, respectively). Moreover, there was no significant decrease in plasma PRL 30 min. after s.c. injection of PCP (10 mg/kg) on the day 29 following 28 days of chronic PCP injection (2.50 + 1.06 ng/ml, n=9). These data suggest the development of tolerance to the PRL-suppressive effect of PCP as a result of long-term administration of the drug. The exact mechanism of the development of this tolerance is unknown, but it might be related to changes in DA receptor sensitivity.

To examine locomotor effect of PCP in these rats, the number of rotations completed in 30.5 x 30.5 cm plastic cylinders was monitored for 30 min. two hrs. after the last of 28 daily injections of PCP. No indications of the development of tolerance was found in these rats, as compared to rats given a single injection of PCP (10 mg/kg, s.c.).

In summary, long-term PCP administration: A. reduces [^{3}H]-spiperone binding to rat striatal membranes; B. produces tolerance to the acute plasma PRL-suppressive effect of PCP; C. does not cause tolerance to acute locomotor-stimulating action of the drug.

290.3 EFFECTS OF TETRAHYDROCANNABINOL ON BRAIN γ-GLUTAMYL TRANSPEPTIDASE. E. Reyes, L. Kaufmann* and B. Jones*. Dept. of Pharmacology, Univ. of New Mexico School of Medicine, Albuquerque, N.M. 87131 and College of Santa Fe, Santa Fe, N.M. 87501.

The effects of oral administration of (-) Δ^9-trans-tetrahydrocannabinol (Δ^9THC) on the membrane bound enzyme, γ-glutamyl transpeptidase (γGTP) was studied. Adult male mice (BALB/cJ) were given an oral dose of Δ^9THC on each of 5 consecutive days in a vehicle of sesame oil, coconut oil and Tween. The dosage ranged from 0.5 to 5 mg/kg. The animals were decapitated and the brains dissected into eight regions. Each brain region was homogenized in Tris buffered saline and assayed for protein and γGTP activity. The higher doses of Δ^9THC produced higher levels of γGTP activity in the brain regions studied. Brain γGTP was partially purified from the group of animals receiving the 5 mg/kg dose of Δ^9THC and the properties compared to those of the control group. Michaelis constants for γ-glutamyl-p-nitroanilide and molecular weights of γGTP in the two groups were determined.

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290.4 TETRAHYDROCANNABINOL EFFECTS ON EXTRAPYRAMIDAL BEHAVIORS: INTERACTIONS WITH PSYCHOACTIVE DRUGS. D. E. Moss, S. B. McMaster*, G. Koob and S. Montgomery*. Department of Psychology, University of Texas at El Paso, El Paso, TX 79968 and The A. V. Davis Center for Behavioral Neurobiology, The Salk Institute, San Diego, CA 92138.

Research reported earlier (Moss, McMaster and Rogers, Pharmac. Biochem. Behav. 15, 779-783, 1981) has shown that delta-9-tetrahydrocannabinol (THC, one of the major psychoactive compounds in marijuana) produces a 20 fold potentiation of reserpine-induced hypokinesia in rats as measured with the bar test. Insofar as reserpine-induced hypokinesia is an animal model of Parkinson's disease and appears to be induced by dopamine depletion in the nigrostriatal system, additional experiments were conducted to study the effects of THC on other dopamine dependent behaviors associated with extrapyramidal function. In these experiments, the effect of THC on methylphenidate- and amphetamine-induced locomotor activity and stereotypies was assessed. Contrary to any simple dopamine specific explanation of THC effects, THC (10 mg/kg by gavage) had no effect on methylphenidate-induced (30 mg/kg i.p.) gnawing but enhanced amphetamine-induced (10 mg/kg i.p.) gnawing. On the other hand, the same dose of THC had no effect on amphetamine-induced (1 mg/kg) locomotor activity but significantly reduced methylphenidate induced (10 mg/kg) locomotor activity.

In other experiments, it has been shown that the effect of THC on reserpine-induced hypokinesia cannot be explained as a THC effect that is observed only in the absence of normal striatal function. In this experiment, striatal function was temporarily interrupted by direct intracranial injection of KCl bilaterally into the caudate/putamen. This treatment caused no hypokinetic effect and, furthermore, additional treatment with THC caused no additional hypokinetic effect. In related experiments, the "cataleptic" effect of THC injected directly into the caudate/putamen (also measured by a bar test) reported by Gough and Olley (Neuropharmacol. 17, 137-144, 1978) could not be replicated. This failure to replicate could, however, be due to differences in bar test procedures used in the different experiments.

The origin of THC potentiation of reserpine-induced hypokinesia does not appear to be related to a direct effect of THC on dopamine function. Our working hypothesis is that produces some effect on extrapyramidal function that cannot be observed behaviorally until dopamine function is blocked in that system.

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290.5 FREQUENCY DEPENDENT EFFECTS OF COCAINE, LIDOCAINE AND d-AMPHETAMINE ON LIMBIC AFTERDISCHARGES. Henry Lesse. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

This study analyzed the comparative effects of cocaine, lidocaine and d-amphetamine on afterdischarges (AD) evoked by low and high frequency limbic stimulation in cats. AD threshold and duration for both 3 Hz and 50 Hz stimulation of the amygdala, dorsal hippocampus and septal region were determined following alternating saline and drug administrations. Cocaine (5 mg/kg), lidocaine (5 mg/kg) and d-amphetamine (2.5-5 mg/kg) were tested in a varied order at 96 hour intervals.

Results indicated a pronounced frequency dependent effect of cocaine on both the amygdala and hippocampus. Following cocaine, afterdischarge thresholds were significantly reduced for 3 Hz stimulation and the opposite effect, an increased threshold, was found when the same brain sites were tested with 50 Hz pulse trains. Septal AD thresholds were unchanged for low frequency stimulation and elevated for high frequency stimulation. In addition, AD duration was consistently reduced at all limbic sites independent of the cocaine-induced increases and decreases in AD threshold. Neither lidocaine nor d-amphetamine resulted in biphasic threshold changes or significant effects on AD duration. Evidence that cocaine-induced changes in AD threshold at identical hippocampal and amygdala sites can be reversed by altering the stimulation frequency suggests that cocaine has a dual excitatory and inhibitory effect, increasing the sensitivity of these limbic structures to repetitive stimulation at low rates while decreasing it at high rates. This differential effect may disrupt frequency coding mechanisms of the amygdala and hippocampus and contribute to the convulsant and anticonvulsant properties of the drug. The absence of similar changes following either d-amphetamine or lidocaine suggests that cocaine's monaminergic and membrane stabilizing (local anesthetic) actions may not a critical determinant of its dual effects on limbic excitability.

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290.6 ACQUISITION OF TOLERANCE TO CAFFEINE'S EFFECTS ON LOCOMOTOR ACTIVITY. H.E. Modrow* and F.A. Holloway. (SPON: A. Revzin) Dept. of Psychiatry and Behavioral Sciences, University of Okla. Health Sciences Center, Oklahoma City, Oklahoma 73190 U.S.A.

We previously reported that caffeine produces an inverted U-shaped dose-effect curve for locomotor activity and also displays rapid acquisition of tolerance or tachyphylaxis (Holloway, et al, Soc. Neurosci. Abst., 7:924, 1981). In this presentation, we examined the acquisition of caffeine tolerance using the same locomotor activity measure (a vibration-sensitive device coupled to a microprocessor) under conditions of chronic drug administration.

Prior to the start of a chronic caffeine regimen, cumulative dose-effect curves for caffeine (10-75 mg/kg), theophylline (10-75 mg/kg), and d-amphetamine (0.1-2 mg/kg) were obtained from male albino rats individually housed with a 12:12 LD cycle. The rats were then injected daily for 66 days with 75 mg/kg caffeine. All the latter injections were given in a single dose with the exception of a weekly cumulative dose regimen. Cumulative dose-effect curves for all drugs were obtained again at the end of the 66 day period. In our prior study we found pronounced locomotor stimulation at moderate caffeine doses in some rats and little or no stimulation in others. Thus, on the basis of the initial cumulative dose-effect curves for caffeine, we divided this study's rats into two groups for analysis. The 3-hour mean activity counts for reactive rats (n=4) was 10-fold higher ($p<.01$) than that for the unreactive rats (n=4) or undrugged controls.

Tachyphylaxis was markedly apparent in the first few weeks of chronic caffeine for both reactive and unreactive rats with cumulative dose days yielding significantly higher activity ($p<.05$) than single dose days. For reactive rats, the cumulative caffeine dosing procedure produced a 2-fold increase in 3-hour activity after Week 1, which subsided to baseline levels by Weeks 3-4. Across weeks the reactive rats displayed significant increases in activity ($p<.05$) after single injections, until by the ninth week no differences in activity were apparent between 75 mg/kg single vs. cumulative dose regimens. The unreactive rats displayed small increases in activity across weeks after both single ($p<.05$) and cumulative ($p<.1$) caffeine doses. Examination of the pre/post chronic caffeine dose-effect curves for theophylline indicated a 2-3 fold increase in activity at the 56 mg/kg dose. A similar comparison for amphetamine activity indicated less stimulation at lower doses ($p<.1$)

In summary, in both caffeine reactive and unreactive rats, chronic tolerance for caffeine's depressant effects on activity develops gradually across weeks. Further, in reactive rats, there is evidence that partial tolerance to caffeine's stimulatory effects on activity develops rather quickly. Whether the residual stimulatory effect eventually would become tolerant is unclear.

290.7 THE INDUCTION OF FIGHTING BEHAVIOR IN MALE MICE AND CHANGES IN BEHAVIORAL RESPONSIVENESS TO PHENCYCLIDINE, AMPHETAMINE AND APOMORPHINE. C.A. Wilmot*, C. VanderWende and M.T. Spoerlein. Rutgers Univ. Coll. Pharm., Piscataway, NJ 08854

Social isolation of male mice produces many behavioral changes, the most prominent being the appearance of fighting behavior towards conspecific males. This behavior is intensified in a dose-dependent manner by low doses of phencyclidine (PCP), 0.75- 2.50 mg./kg., i.p., when tested at 6 weeks of isolation, as previously described. (Soc. Neurosci. Abs., 1981). A repeated observation in our lab has been that PCP does not increase the percentage of animals fighting, however the intensity of those fighting is significantly greater than vehicle-treated isolates. Only infrequently (less than 5% of all observations) has PCP been seen to release fighting behavior in group-housed mice. Thus PCP appears to exaggerate this behavior only in those mice already predisposed to fight through isolation.

We have examined the effects of isolation on two other behavioral responses, amphetamine-induced locomotor activity and apomorphine-induced climbing behavior, to consider the hypothesis of an increased dopaminergic sensitivity of isolated mice as a predisposing factor. Vehicle-treated isolates show a greater baseline locomotor activity and climbing behavior. Also, the dose-response curves for isolated mice show an increased responsiveness to low doses when compared to group-housed age-matched controls. These results suggest that isolation may induce a state of autoreceptor hyposensitivity.

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290.8 DOSE-DEPENDENT BEHAVIORAL CHANGES IN SELECTED MEMBERS OF A PRIMATE SOCIAL COLONY FOLLOWING ADMINISTRATION OF THE HALLUCINOGEN 3,4-METHYLENEDIOXYAMPHETAMINE (MDA). W.J. Heinze*, R.F. Schlemmer, Jr., & J.M. Davis. Illinois State Psychiatric Institute, Chicago, Illinois 60612 & National College, Lombard, Illinois 60148.

3,4-Methylenedioxyamphetamine (MDA) is a hallucinogen which attained great street popularity during the last decade. MDA was proclaimed a mild psychedelic and hence was coined "Mellow Drug of America". Pharmacologically, MDA is of interest because of its structural similarity to both mescaline and amphetamine. Despite the clinical and theoretical interest in MDA, relatively few studies have investigated the behavioral pharmacology of this drug. The purpose of this study was to test the behavioral effects of four acute doses of MDA on four adult members of a primate social colony. Following a 16-day baseline observation period, during which saline was injected i.m. to all members of a stable primate social colony of 4 adult Stumptail macaques, one dose of MDA was administered i.m. to one animal per day 15 minutes prior to observation while the 3 untreated animals received saline at the same time. By the completion of the study, all animals had received four doses of MDA (0.3, 1.0, 3.0, & 10.0 mg(base)/kg) in a latin-square design. At least two weeks separated each drug treatment for an individual monkey. All observations were conducted by the same experienced "blind" observer, using the focal sampling technique. Two emergent behaviors, limb jerks and body shakes, were induced by MDA. MDA induced a dose-dependent increase in limb jerks (a myoclonic spasm of an extremity) at doses of 1 mg/kg & larger. Lower doses of MDA increased the frequency of total body shakes, whereas larger doses decreased them. MDA significantly increased the frequency of checking over baseline in a dose-dependent manner. MDA also increased the distance between treated and untreated monkeys, when compared to baseline, in a dose-dependent fashion. Both social grooming and self grooming were eliminated at doses of 1.0 mg/kg and larger. MDA increased the frequency of submissive gestures given by treated monkeys above baseline levels at the lower doses tested. Interestingly, treated animals were approached by control monkeys more frequently than during baseline. The results of this study demonstrate that MDA, in a dose range similar to that used by humans, induces profound changes in primate social and solitary behavior. The pattern of behavioral changes induced by MDA closely resembles that induced in this species by other hallucinogens including LSD, DOM, mescaline, and DMT. These changes include the induction of limb jerks and body shakes as emergent behaviors, a reduction or elimination of both social and self grooming, increased distancing between treated and control animals - suggestive of social withdrawal, and increased checking.

290.9 PCP ANALOGUES AS POTENTIAL PCP ANTAGONISTS. E.C. C. Si*, D. Nichols, M. P. Holsapple* and G. K. W. Yim. (SPON: L. J. Pellegrino). Dept. Pharmacology and Toxicology, Dept. Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Science, Purdue Univ., West Lafayette, IN 47907.

Of a series of PCP (phencyclidine) analogues, the cis and tran-N-(1-phenyl-4-methylcyclohexyl) piperidine had comparable or higher affinity to the PCP receptor. The lesser activity of the PCP analogues on the rotarod test (Vincent et al., 1979) prompted this evaluation of these PCP analogues as potential antagonist of various actions of PCP.

In the isolated frog sciatic nerve, the trans isomer at 10^{-3}M produced the largest depression of the amplitude of action potential of the nerve (94±3%), followed by PCP (84±3%) and cis isomer (48±2%). The cis isomer, at 10^{-4}M, did not depress the action potential, but did attenuate the depression by PCP by half ($p<0.05$). In the field stimulated rat ventricular strip, all the three compounds, i.e. trans isomer, PCP and cis isomer, produced dose-related increases in the amplitude of the strip contraction, with their peak increases being 56.7±8.4%, 44.5±8.5%, and 25±4.2%, respectively. However, the presence of the cis isomer at 10^{-6}M, which by itself had little effect on the contraction of the strip, failed to reduce the positive inotropic effect of $3X10^{-5}$M and 10^{-4}M PCP. In the rotarod test in mice, the ED_{50} of PCP, trans isomer and cis isomer were 4.7 mg/kg, 12.1 mg/kg and 56 mg/kg, respectively. The cis isomer, at either 10 mg/kg or 30 mg/kg, failed to decrease the ataxic effect of ED_{80} dose of PCP. In mice, the 24-hour LD_{50} values for PCP, trans isomer and cis isomer were 67 mg/kg, 71 mg/kg and >150 mg/kg respectively. Again, the non-ataxic, non-lethal dose of the cis isomer (19 mg/kg) failed to reduce the lethality caused by the LD_{80} dose of PCP.

In summary, the cis isomer was a partial agonist of PCP in the 4 preparations studied. Although the cis isomer did reduce the nerve blocking action of PCP, it failed to antagonize the myocardial stimulant, ataxic and lethal actions of PCP. (Supported in part by USPHS grant DA 02327).

290.10 INTRACRANIAL COCAINE SELF-ADMINISTRATION. N.E. Goeders and J.E. Smith, Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Cocaine is a local anesthetic agent which is also one of the most reinforcing of the drugs of abuse. This investigation was initiated to determine the neuronal circuitry mediating cocaine reinforcement processes using intracranial self-administration (ICSA) methodologies. Experimentally naive male Fisher F-344 rats were stereotaxically implanted unilaterally with guide cannulae into either the nucleus accumbens, ventral tegmental area, sulcal prefrontal cortex or medial prefrontal cortex. The animals were allowed to self-administer 100 nl microinjections of cocaine HCl directly into the brain on alternate days for eight hours by depressing a lever located on one wall of the chamber. The microinjections were delivered by an adaptation of the electrolytic microinjection transducer (EMIT) system described by Bozarth and Wise (Life Sci. 21, 639, 1981). Concentrations of 50 to 5000 pmoles of the drug dissolved in artificial CSF were tested for ICSA into the various brain areas. Cocaine did not maintain responding for its direct microinjection into the nucleus accumbens or the ventral tegmental area. On the other hand, animals were rapidly trained to self-administer cocaine into the medial and sulcal prefrontal cortices at rates significantly higher than vehicle alone (vehicle = 5 ± 1; cocaine = 18 ± 2). Dose response experiments indicated that 100 pmoles of the drug resulted in the most consistent ICSA. The reinforcing properties of these cortical microinjections were further strengthened by the demonstration of cocaine ICSA into the medial prefrontal cortex on intermittent schedules of reinforcement. In addition, the animals responded significantly more often on the active lever than on an inactive lever installed on the opposite wall of the chamber. Not all areas of the medial prefrontal cortex supported cocaine ICSA, suggesting the involvement and importance of a specific subpopulation of neurons. Indirect evidence has indicated that the mesolimbic dopaminergic neuronal system is involved in cocaine reinforcement processes. 6-OHDA lesions of the nucleus accumbens attenuated intravenous cocaine self-administration (Roberts et al, Pharm. Biochem. Behav. 12, 781, 1980). Manaco et al (Neurobiol. of Nucleus Accumbens, 338, 1981) has also reported amphetamine ICSA into the nucleus accumbens. However, the results of this investigation clearly demonstrate that cocaine microinjections into the medial prefrontal cortex are reinforcing while microinjections into the nucleus accumbens or ventral tegmental area are not. These data suggest that cortical rather than subcortical structures are intimately linked to cocaine reinforcement processes. (Supported in part by Pharmaceutical Manufacturers Association Foundation Advanced Predoctoral Fellowship and by USPHS Grant DA-01999-04).

290.11 EFFECTS OF CIGARETTE SMOKING AND MONETARY REINFORCED RESPONDING ON SALIVA CORTISOL LEVELS. D.R. Cherek, J.D. Lane and J.E. Smith. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Several studies have reported elevated cortisol levels associated with cigarette smoking. However, most of these studies have required subjects to smoke high nicotine cigarettes and/or several cigarettes within relatively short periods of time. The following study sought to determine the effects of a 2-hour daily monetary reinforced operant task on cortisol levels in the presence and absence of cigarettes. Subjects smoked their preferred brand of cigarettes and were allowed to regulate their own smoking. Cortisol levels were measured in saliva before and after each daily experimental session. The saliva cortisol levels were compared within the same subject during four different conditions: (1) no smoking + no task, (2) smoking + no task (3) no smoking + task, and (4) smoking + task. The experiment determined if cigarette deprivation of 2-1/2 hours (i.e., a 30-minute waiting period and 2-hour session) produced an elevation of cortisol levels, indicating significant stress either in the absence or presence of the work task. Also, the effects of cigarette smoking on cortisol levels in the absence and presence of the task were determined. The operant task consisted of a 2-hour session of responding to accumulate monetary reinforcement, requiring visual monitoring of a stimulus light. Four male research subjects participated in the experimental sessions five days per week, and were randomly exposed to the four conditions over successive sessions. There were no differences observed in the saliva cortisol levels in the no smoking condition (subject simply set in the experimental room for two hours) or the no smoking task condition. Thus the cigarette deprivation did not affect saliva cortisol level. The simulated work task also did not affect saliva cortisol level. There was no difference between the smoking condition (subject set in the experimental room for two hours and was allowed to smoke), and the smoking task condition. The amount of cigarette smoking also did not influence saliva cortisol levels. Most subjects increased the number of cigarettes smoked and/or number of cigarette puffs when they were also performing the operant task. This increase in cigarette smoking compared to the smoking no task condition did not change the saliva cortisol level. Thus, cigarette smokers who are allowed to smoke their preferred brand of cigarettes at their own rate, do not show changes in cortisol levels. The implication that cortisol elevations during smoking may play a role in the health risks associated with smoking and the reinforcement mechanism for smoking via association with ACTH and β-endorphin release were not supported.

290.12 NUCLEUS ACCUMBENS LESIONS SELECTIVELY ATTENUATE COCAINE BUT NOT HEROIN SELF-ADMINISTRATION IN RATS. H.O. Pettit*, A. Ettenberg, F.E. Bloom and G.F. Koob. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, San Diego, California 92138. (SPON: L.Y. Koda)

Recent reports have suggested an involvement of the mesolimbic dopamine (DA) system in the reinforcing actions of both opiate and stimulant drugs. However, previous data from our laboratory has demonstrated that pharmacological antagonism of the DA system selectively alters cocaine but not heroin self-administration in rats. Conversely, opiate antagonist drugs affect only heroin-reinforced behavior. In the present study, the role of mesolimbic DA neurons in drug reinforcement was further examined by testing the effects of lesioning the DA terminals in the Nucleus Accumbens Septi (NAS) of rats trained to self-administer both cocaine and heroin, on alternate days.

Male albino rats were implanted with chronic jugular catheters and stereotaxic injection cannulae aimed at the NAS. Following surgery each rat was allowed to lever-press in their home cages for intravenous injections of heroin (60 ug/kg/infusion) every other day. On alternate days, the same animals were allowed to respond on a different lever for intravenous cocaine (0.75 mg/kg/infusion). Drug access was limited to a 3 hr session each day. Once responding for both drugs had stabilized for a given animal, bilateral 8 ug injections of the neurotoxin 6-hydroxydopamine were administered through the NAS cannulae. Following three days of recovery, the alternating self-administration schedule was resumed. On the first post-lesion test day, both heroin and cocaine intake was suppressed. However, by the end of the first week of post-lesion testing, heroin self-administration had returned to pre-lesion rates while both the rate and pattern of cocaine self-administration was still disrupted. This disruption in cocaine-reinforced responding was still evident 13 days post-lesion.

The present study demonstrates that selective lesions of the DA terminals in the Nucleus Accumbens interfere with stimulant but not opiate self-administration. These results are, therefore, consistent with the notion that separate neural substrates mediate the reinforcing actions of cocaine and heroin.

This research was supported by federal grant NIDA 01785.

290.13 EFFECTS OF LSD ON SENSORY EVOKED ACTIVITY IN POLYSENSORY AREAS OF CAT BRAIN. D.M. Wilkison* and M.J. Hosko. Lab. of Neuropharmacology, Medical College of Wisconsin, Milwaukee, WI 53226.

Effects associated with LSD intoxication include disruption of visual perception, overt hallucination and a mixing of sensory experience or synesthesia. The effects of LSD on sensory evoked activity in cortical and subcortical areas which receive multimodal sensory information was investigated as a possible site of action of the sensory cross-over induced by LSD. Acutely prepared α-chloralose-anesthetized cats were used. The effects of LSD (25-200 μg/kg) were evaluated in primary and multimodality areas with visual (optic chiasm stimulation) and somatic (radial nerve stimulation) sensory stimulation. Single doses of LSD produced a dose-dependent depression of visual evoked response at LGN and area 18, $F(3,64) = 13.4$; $P < .001$ reaching 60% depression of the amplitude of the evoked potential in area 18. In the same cats the primary somatosensory response was facilitated $F(1,68) = 6.5$, $P < .005$. The response in the multimodal area, anterior marginal gyrus, to both visual and somatosensory evoked activity was depressed by LSD up to 80%. The visual response was more sensitive, 50% inhibition at 50 μg/kg, than the somatic response, 50% inhibition at 100-150 μg/kg. Multimodal responses in N. central median tended to follow those of the anterior marginal area. Transcallosal activation of the anterior marginal cortex by electrical stimulation of the contralateral anterior marginal cortex was not altered by LSD. The differential effects of LSD on primary and multimodal sensory pathways was further investigated using cyproheptadine (CYP) and methysergide (MET). CYP, 1 mg/kg, blocked the effects of LSD on primary and multimodal pathways. MET, 1 mg/kg, administered alone facilitated visual primaries, but when administered with LSD potentiated the LSD-induced depression, especially of the visual responses. These data suggest LSD has effects on primary sensory responses which are mediated by 5-HT receptor systems and are independent of its effects on polysensory responses. The greater sensitivity of the visual response in the polysensory areas to LSD could be accounted for by the additional depression in the primary path (LGN) which would reduce activation of the polysensory areas. The effects of 5-HT antagonists suggest LSD actions were mediated by a combination of S1 and S3 receptors according to the scheme of Aghajanian. It is of interest that MET, a mild hallucinogen in man, potentiated the effects of LSD on polysensory information processing. (Supported by PHS Grant R01 DA 01754).

290.14 EFFECTS OF CANNABINOIDS ON BINDING TO DOPAMINERGIC RECEPTORS IN THE MOUSE BRAIN. Alan S. Bloom, Theresa M. Dieringer* and Janice K. Greathouse*, Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

We have previously reported that Δ^9-Tetrahydrocannabinol (THC) and its active metabolite, 11-OH-THC produced a biphasic increase in the binding of ^{3}H-DHA to β-receptors in the mouse brain which was characterized by an increase in the apparent affinity (decreased K_d) for the ligand (Hillard and Bloom, Brain Res. 235: 370, 1982). Cannabidiol (CBD), a cannabinoid without marijuana-like psychoactivity, was inactive in this system. In order to determine the specificity of this phenomena, we have now examined the effects of these cannabinoids on the binding of the dopaminergic antagonist ^{3}H-spiroperidol (SPIR) and the agonist ^{3}H-ADTN to receptors in the mouse corpus striatum. In vitro, THC concentrations between 1 and 100 μM produced a dose related inhibition of the binding of SPIR. Binding in the presence of 30 μM THC was reduced to 69% of the vehicle control value. A similar inhibition of binding was observed after treatment with either 30 μM concentrations of 11-OH-THC or CBD. Saturation binding studies indicated that there was a significant increase in the K_d produced by the 30 μM concentration of THC when compared to vehicle treated preparations. The mean K_d increased from .14 nM to .23 nM after THC treatment. There was no change in the B_{max}. Mice were also treated either acutely or chronically with increasing doses of THC for up to 6 days. No change was observed in the binding of SPIR in the brains of these animals when compared to vehicle treated controls. Similar effects of the cannabinoids were observed on the binding of the dopaminergic agonist ^{3}H-ADTN. ADTN binding was reduced to 62% of the vehicle control level by a 30 μM concentration of THC. 11-OH-THC was equipotent with THC. CBD was significantly more potent than THC. Binding in the presence of a 30 μM concentration of CBD was only 27% of that observed in the presence of vehicle alone. As was the case with SPIR binding, in vivo treatment with either THC or CBD had no effect on ADTN binding. The results of these studies indicate that in vitro, several cannabinoids can inhibit the binding of both dopaminergic agonists and antagonists to their receptors. However, in vivo treatment with the cannabinoids appears to be without effect on these systems. Furthermore, the effects of the cannabinoids on these receptors is quite different than on β-adrenergic receptors suggesting that there is some specificity of cannabinoid action at the neurotransmitter receptor level. (Supported by USPHS Grant DA 00124).

290.15 DIFFERENTIAL EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON THE BINDING OF AGONISTS AND ANTAGONISTS TO THE β-ADRENERGIC RECEPTOR. Cecilia J. Hillard and Alan S. Bloom. Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

We have demonstrated that Δ^9-tetrahydrocannabinol (THC), administered in vitro, increases the binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) to the β-adrenergic receptor of cerebral cortical membranes (Hillard, CJ and Bloom, AS, Brain Res., 235: 370, (1982). The increase in ^{3}H-DHA binding is due to an increase in affinity with no change in binding site density (Bmax). We have now investigated the effects of in vitro THC on the competition of other ligands with ^{3}H-DHA for β-adrenergic binding sites.

Male ICR mice were used in these studies. Cerebral cortical membranes were prepared as previously described. THC was administered using an emulphor-ethanol-buffer vehicle. K_I values were calculated from IC_{50}'s using the formula of Cheng and Prusoff (Cheng, YC and Prusoff, WH, Biochem. Pharm., 22: 3099, 1973).

The effects of 10 μM THC on the competition of agonists and antagonists with 7 nM ^{3}H-DHA are shown below.

Competing Ligand	K_I Vehicle	K_I 10 μM THC	Pseudo-Hill Coeff. (n) Vehicle	Pseudo-Hill Coeff. (n) 10 μM THC
Propranolol	19.1 nM	4.03 nM	1.10	0.73
Alprenolol	4.65 nM	0.736 nM	0.91	0.64
Practolol	7.69 μM	1.30 μM	0.76	0.50
Sotalol	0.359 μM	0.089 μM	0.54	0.49
Dichloro-isoproterenol	21.2 nM	20.6 nM	0.50	0.59
Isoproterenol	0.10 μM	0.208 μM	0.63	0.48
Norepinephrine	1.09 μM	1.33 μM	0.47	0.59
Epinephrine	0.88 μM	1.17 μM	0.64	0.49

THC decreased the K_I's of antagonists, analogous to the change produced in ^{3}H-DHA binding. The affinity of antagonists was increased regardless of lipid solubility: log P of propranolol is 3.65 while log P of sotalol and practolol are -0.79 and 0.79, respectively (Cruickshank, JM, Amer. Heart J., 100: 160, 1980). In addition, the pseudo-Hill coefficients of antagonists were reduced by THC. Conversely, THC slightly increased the K_I values of agonists and had no consistent effect on the pseudo-Hill coefficients. The partial agonist dichloroisoproterenol was apparently unaffected by THC. Therefore, THC has very different effects on the binding of agonists and antagonists to the β-adrenergic receptor. (Supported by USPHS grant DA-00124).

290.16 ADENOSINE ANALOGS ANTAGONIZE PHENCYCLIDINE DISCRIMINATION. Ronald G. Browne, Pfizer Inc., Groton, CT 06340.

Phencyclidine (PCP) is a psychotomimetic which has been reported to produce long lasting psychosis indistinguishable from schizophrenia. Because of the close similarity between PCP-induced and endogenous psychoses, novel compounds capable of antagonizing PCP's effects might be useful as antipsychotic agents. The present investigation examined the ability of various agents to mimic or block the behavioral effects of PCP in a drug discrimination paradigm.

Male Sprague-Dawley rats were trained to discriminate 3.2 mg/kg of PCP from vehicle in a two-lever operant procedure. Discrimination accuracy was established following about 40 training sessions as evidenced by most animals emitting their first unreinforced FR-10 responses on the appropriate lever in nine out of ten consecutive sessions. Generalization testing indicated that ketamine, dexoxadrol, tiletramine, SKF10047, and PCP analogs mimiced PCP's subjective effects, in contrast to a number of agents including LSD, morphine, and psychomotor stimulants which were discriminated as vehicle.

When the rats were pretreated with various psychotropic agents, including neuroleptics, no significant alteration in PCP discrimination was observed. In contrast, when pretreated with the adenosine receptor agonists N6-cyclohexyladenosine or L-phenylisopropyladenosine (LPIA), the discriminative properties of PCP were completely blocked. The D-isomer of PIA, which is 30 to 100 times weaker as an adenosine agonist, was likewise less potent in blocking the PCP cue. The blocking effect of the adenosine agonists could be prevented by simultaneous administration of theophylline.

The results of these studies, which suggest that potent adenosine receptor agonists might be useful in treating PCP-induced psychosis, will be discussed in relation to interactions between PCP and adenosine agonists at their respective receptor binding sites.

290.17 DIFFERENTIAL EFFECTS OF THE MAO INHIBITOR PHENELZINE ON BEHAVIORAL CHANGES INDUCED BY d-LSD & 5-METHOXYDIMETHYLTRYPTAMINE IN SELECTED MEMBERS OF PRIMATE SOCIAL COLONIES. R.F. Schlemmer, Jr., G.N. Pandey, C.B. Tyler, N. Narasimhachari, W.J. Heinze*, and J.M. Davis. Illinois State Psychiatric Institute and University of Illinois, Chicago, Illinois 60612 and Med. Coll. of VA, Richmond.

d-Lysergic acid diethylamide (LSD) & 5-methoxydimethyltryptamine (5-MeODMT) are potent hallucinogens in humans and induce many similar behavioral changes in animals. LSD and 5-MeODMT induce similar changes in several primate social and solitary behaviors including limb jerks (myoclonic spasms of the extremities) which are specifically induced in this species by hallucinogens. Several reports suggest that the effects of LSD and 5-MeODMT may be differentiated by concomitant administration of monoamine oxidase (MAO) inhibitors. The purpose of the present study was to compare the effect of the MAO inhibitor, phenelzine (PHZ), on the behavioral changes induced by LSD and 5-MeODMT in selected members of primate social colonies. The subjects for the study were members of 2 stable, adult Stumptail macaque social colonies of 5 members each. One colony was studied for each drug. Following an initial observation period where baseline behavioral scores were determined, individual members of each colony received treatment with 4 doses of 5-MeODMT (0.05-0.25 mg/kg) or 5 doses of LSD (0.0003-0.03 mg/kg) over a 10 wk. period. This was followed by 2 treatment periods with PHZ, 4 mg/kg/day in 2 divided doses, for 14 days with 5-MeODMT (0.05 or 0.10 mg/kg) or LSD (0.01 or 0.03 mg/kg) concomitantly given during the final 5 days. All drugs were given i.m. or n.g. MAO inhibition was determined in all PHZ-treated monkeys prior to administration of the hallucinogen by assaying platelet MAO activity using ^{14}C-tyramine as substrate. All animals had MAO inhibition of greater than 93% prior to administration of the hallucinogen. A 60 min. behavioral observation period was conducted daily by a "blind" experienced observer who quantified and recorded the behavior of each animal in the colony using the focal sampling technique. LSD and 5-MeODMT both induced dose-dependent increases in limb jerks, body shakes, and checking behavior (visual scanning) and a reduction in social grooming. PHZ significantly potentiated 5-MeODMT-induced limb jerks, body shakes, & increased checking and 5-MeODMT-induced disruption of social grooming. Conversely, PHZ significantly antagonized LSD-induced limb jerks, body shakes, and increased checking, but only weakly antagonized LSD-induced disruption of social grooming. The results of this study demonstrate differential effects of the same MAO inhibitor on 3 identical behavioral changes induced by 2 hallucinogens in the same primate species. Since it is difficult to reconcile these findings in terms of any present theories of hallucinogenic drug action, a reconsideration of existing theories may be indicated.

290.18 THE EFFECT OF REPETITIVE DOSES AND HORMONAL STATUS ON SPECIFIC AMPHETAMINE-ELICITED BEHAVIORAL RESPONSES IN THE MALE RAT. D. Dluzen, M. Green and V. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, Il 61801.

The behavioral response of male rats to 4 doses of d-amphetamine sulfate (AMPH) as well as the effect of castration and testosterone replacement on these behaviors in response to this psychostimulant were examined. The intensity/duration scores of the behaviors, gnawing, grooming, head bobbing, licking, rearing, stationary, sleeping and sniffing were recorded for 30 consecutive 5 minute intervals starting within 5 minutes after injection. For each interval a score (0-3) reflecting the intensity/duration was assigned to each behavior; scores were totaled over the 30 intervals and expressed as the % of maximal possible score (90). In Experiment I, 4 doses of AMPH (2.5, 5, 10 and 15 mg/kg; ip) with 4 intact male rats/dose were used. Three distinct response categories were observed. In the first, dose dependent increases (X± SE for respective doses) were obtained for sniffing (45.3±7.4, 52.3±3.9, 55.2±5 and 59.4±9.3 %), stationary (68.8±8.6, 75.8±6.6, 86.6±2.4 and 90.1±1.2 %) and grooming (.5±.3, 3.1±3.1, 24.0±14.5 and 38.3±7.9 %). In the second, dose independent responses or no changes were obtained for head bobbing (9.9±6.6, 13.8±4.1, 9.1± 4.5 and 21.4±15.5 %), sleeping (11±2.4, 1.3±.8, 0±0 and 1.6±1.6%) and licking (0±0, 0±0, 23.5±12.6 and .8±.5 %). In the final category relatively high levels in males receiving 2.5 and 5 mg/kg and significant reductions with the 10 and 15 mg/kg doses were obtained for rearing (41.2±14.3, 43.2±8.8, 18.0±5.1 and 2.4±1.7 %) and grooming (14.4±4.7, 31.3±16.4, 3.1±.4 and 1.3±1 %). In Experiment II the effect of AMPH (5 mg/kg) on these behaviors in long term castrate (75 days) rats receiving oil or testosterone propionate (TP-100ug/100gm bw; sc) was examined. Following daily TP or oil injections, males (4/group) were tested at 4 weekly intervals with AMPH and one control saline test(SAL). Mean AMPH rearing scores (collapsed over 4 AMPH tests) of TP males were significantly greater than that following SAL (47.2±9.7 vs 7.3±2 %) and from that of oil males (13.9±4 %). Grooming scores of TP males were elevated over the 4 AMPH tests while that of oil males demonstrated a significant decrease after the first AMPH test and were overall lower than TP males (23.2±7.9 vs 8±4.2 %). The remaining behaviors demonstrated no significant differences over AMPH tests or as a function of TP or oil treatment and were significantly greater during AMPH vs SAL, with the exception of sleeping in which SAL>AMPH (TP=55.8 vs 0 and Oil=69.3 vs 0 %) and gnawing which was not observed following AMPH or SAL. These results illustrate that behavioral effects of AMPH are complex, depending upon dose and specific behavior considered. Moreover, AMPH stimulated responses of specific behaviors can be altered as a function of hormonal status and/or repetitive administration.

290.19 INTRAVENOUS NICOTINE SELF-ADMINISTRATION BY HUMANS AND TOLERANCE TO SUBJECTIVE EFFECTS OF NICOTINE. J.E. Henningfield*, K. Miyasato* and D.R. Jasinski. NIDA Addiction Research Center, Baltimore MD 21224.

Whether nicotine can function as a reinforcer for humans and thus strengthen behavior leading to its self-administration is a question fundamental to the understanding of tobacco dependence. Previous studies have shown that intravenous nicotine produces many of the effects produced by smoking cigarettes. In the present study, nicotine (0.75 to 4.5 mg per injection) and saline injections were delivered via an indwelling catheter placed in the forearm vein of male volunteer cigarette smokers. Completion of a fixed number of lever presses produced an injection, and simultaneously, activation of a tone and a light. Two finger-operated levers provided a choice between saline and nicotine. The drug and saline levers were alternated each session on a double-blind basis. The procedures, similar to those used in studies of drug self-administration by animals, were supplemented by pre- and post- session evaluation of mood and feeling states using psychometric instruments previously shown to be sensitive to drug induced changes. Cigarette smoking was not permitted for 1 h before, nor during, the 3 h sessions. All 15 subjects were found to self-administer nicotine. Nicotine injections were regularly spaced, and the temporal patterns were similar to those observed for humans smoking cigarettes and for animals injecting stimulants under analogous conditions. When nicotine dose per injection was varied across sessions, total nicotine intake (mg/3h session) was directly related to injection dose while number of injections was inversely related. At higher dose levels, the nicotine lever was consistently preferred to the saline lever. Furthermore, nicotine produced dose-related increases in post-session drug liking scores. Mecamylamine pretreatment attenuated the subjective effects of nicotine and produced saline-like patterns of self-administration. In one experiment, subjects rated pleasureable (euphoric) and unpleasurable (dysphoric) effects of nicotine and saline by placing marks on 100mm line visual analogue scales 1 min after each injection. Maximum euphoric effects were produced by the initial nicotine injections and decreased across subsequent injections, while dysphoric effects increased with successive injections. Saline injections produced negligible effects. Injection rates declined when euphoric effects became weak. These results indicate that iv nicotine may serve as a positive reinforcer and that patterns of self-administration are related to the subjective effects produced by the drug. Finally, these results support the hypothesis that nicotine may serve as a reinforcer for the behavior of cigarette smoking, thus clarifying the fundamental commonality between cigarette smoking and other forms of drug dependence.

290.20 WHOLE-BODY AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-LABELLED PHENCYCLIDINE IN MICE. I. Fand, D.G. Deutsch, W.P. McNally*, P. Som*, Y. Yonekura* and A.B. Brill*. L.I. Res. Inst., and Dept. of Pathology, SUNY at Stony Brook, Stony Brook, NY 11794 and Medical Dept., Brookhaven Natl. Lab., Upton, NY 11973.

Phencyclidine (PCP) is a drug of abuse having profound pharmacological effects. Recently, considerable interest has been focused on PCP's unique action as a schizophrenomimetic agent. The present study was designed to better understand the mechanisms underlying PCP intoxication by providing detailed information on the disposition of (^{3}H)-PCP in the whole animal.

The existence of specific receptor sites for PCP in rodent brain has been previously documented by in vitro binding studies (Vincent et al., PNAS 76:4678, 1979) and by study of (^{3}H)-PCP binding to slide mounted sections of fresh frozen rat brain (Quirion et al., PNAS 78:5881, 1981). The present investigation extends earlier studies and describes the localization of (^{3}H)-PCP in vivo by employing a macroautoradiographic technique which avoids possible sources of error which may be introduced by preselection of central and peripheral tissues obtained by dissection.

Adult male Hale-Stoner strain BNL mice were administered a single dose of 250 µCi 1-(1-[phenyl-3-^{3}H]cyclohexyl) piperidine (17 Ci/mM) by tail-vein injection in 0.2 ml of a saline solution. Animals were sacrificed by immersion in liquid N_2 at intervals of 1,2,3,4,5,20,60 and 120 minutes and prepared for whole-body sectioning. Sections were cut at 50 µm, freeze-dried and affixed to LKB Ultrofilm for exposure. After development, autoradiographic densities were determined densitometrically.

Computer-assisted densitometric analysis showed that (^{3}H)-PCP radioactivity was very rapidly removed from the blood and distributed to central and peripheral sites. (^{3}H)-PCP radioactivity located to the adrenal medulla as early as five minutes and persisted in this tissue as long as 60 minutes after injection. Distribution of (^{3}H)-PCP radioactivity was essentially uniform in the brain at five minutes after dosing. Later distribution intervals showed label localization to frontal cortex, striatum and hippocampus. The fundic stomach was identified as a major route of (^{3}H)-PCP excretion for periods up to 120 minutes after injection. At the 20-minute survival time interval the relative order of decreasing (^{3}H)-radioactivity was found to be: kidney, liver, salivary gland, adrenal medulla, lungs, spleen, myocardium, brain, blood and striated muscle.

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